ISK2019

8th International Symposium on Kallikreins and Kallikrein-Related Peptidases

Prague, 25 – 27 September 2019

Final Programme & Abstract Book

8th International Symposium on Kallikreins and Kallikrein-Related Peptidases,
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We thank to all our supporters and sponsors for their contribution to the symposium

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WELCOME MESSAGE

Dear Colleagues,

It is our great pleasure to welcome you to the 8th International Symposium on Kallikreins (KLK) and Kallikrein-Related Peptidases (ISK2019), which is held at the Institute of Molecular Genetics of the Czech Academy of Sciences in Prague.

The 8th edition of this international symposium offers a multidisciplinary programme of presentations on the latest developments, covering all areas of kallikrein research and clinical applications. A major theme of this symposium highlights the utility of genetic models to unravel the role of kallikrein proteases in healthy, and diseased physiology. The topics range from themes such as KLK genetics and genomics to KLKs in physiology and pathophysiology: e.g. skin and brain disorders, inflammatory and infectious diseases, and KLKs in malignant diseases using biomarkers and pathophysiology. Other interesting topics to be covered are: KLKs in tissue and cell signaling, the structural and functional aspects of Kallikreins (substrates and inhibitors), and the application of therapeutic approaches and drug design.

The programme of the Prague meeting includes a wide range of invited speakers, short talks and seminars from early career researchers, as well as a poster session. An integral part of the programme will be traditional lectures sponsored by the Frey-Werle and Henner Graeff foundations.

Prague - the capital of the Czech Republic - is situated on the Vltava River and has always been an important crossroad of trade and culture in Europe. Prague is often called "Golden" or "Hundred-spired", and belongs to the architecturally unique European cities famous for a mix of all architectural styles. Romanesque rotundas, Gothic cathedrals and Baroque and Renaissance palaces are all featured in Prague's magnificent skyline, as well as buildings influenced by the Art Nouveau, Classicist, Cubist and Functionalist styles, and modern buildings.

The main attractions include the Prague Castle, the Charles Bridge, Old Town Square with the Prague astronomical clock, the Jewish Quarter, the Petřín hill and Vyšehrad. Not surprisingly, the historic centre of Prague is on the UNESCO list of World Heritage Sites.

Last but not least, we would like to thank the members of the International Scientific Advisory Committee for their invaluable contribution to the programme of the symposium. We would also like to thank our sponsors for their generous support of ISK2019.

We hope you will enjoy the ISK2019 symposium.

Yours sincerely,
Programme and Local Organizing Committee

E.K. FREY – E. WERLE FOUNDATION OF THE HENNING L. VOIGT FAMILY & HENNER GRAEFF FOUNDATION

The E.K. Frey - E. Werle Foundation (http://www.frey-werle-foundation.com/Activities.html) is named after Emil Karl Frey and Eugen Werle, two scientists who discovered the kallikrein-kinin system, and the Henning L. Voigt Family, who donated 1 Million DM for its establishment in 1988. The purpose of the Foundation is the promotion of science to clarify the role of the kallikrein-kinin system and related subjects in health and disease.

The HENNER GRAEFF Foundation (http://henner-graeff-stiftung.de/en/) honors Prof. Henner Graeff, MD, who was Chairman and Head of the Department of Obstetrics and Gynecology at the Technische Universität München from 1982 to 2000. Aims of the HENNER GRAEFF Foundation are promotion of science and research in the field of tumor cell biology/oncology.

The E.K. Frey - E. Werle and HENNER GRAEFF Foundations allocate Awards to scientists who have made outstanding contributions in the fields of these Foundations.

- **The Commemorative Gold Medal** is awarded to scientists who have made outstanding contributions to our knowledge of the role of the kallikrein-kinin system and related subjects in health and disease over decades, so that their research achievements have influenced the developments in the field.

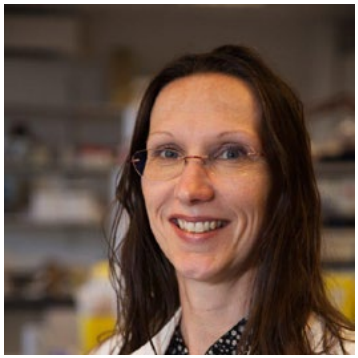
- **The Promotion Prize** is given jointly by the two Foundations and is awarded to young scientists presenting outstanding contributions to contemporary research promoting further progress in view of the aims of the Foundations.

- **The Young Investigator Award** is awarded to junior scientists presenting new impressive results with high significance for the aims of the Foundations.

E.K. Frey - E. Werle Foundation of the Henning L. Voigt Family

HENNER GRAEFF Foundation

The 2019 Laureates are



E.K. Frey – E. Werle and Henner Graeff Promotion Prize

Daniela LOESSNER
Barts Cancer Institute, London, United Kingdom

"Kallikrein-related peptidases are key elements in the tumour microenvironment"

E.K. Frey – E. Werle Young Investigator Award

Srilakshmi Srinivasan
Queensland University of Technology, Australia

"Prostate cancer risk associated Single Nucleotide Polymorphism affects PSA glycosylation and its function"

Henner Graeff Young Investigator Award

Amiram Sananes
Ben Gurion University, Israel

"Combinatorial Engineering of APPI Variant with Improved Proteolytic Resistance and KLK6 Selectivity for Cancer Imaging and Therapy"

Wednesday
25 September 2019

10:00 - 10:30	Opening
10:30 - 11:15	01 - KLK genetics and beyond (genetics, genomics, other 'omics' technologies)
11:15 - 11:45	Coffee break
11:45 - 12:30	01 - KLK genetics and beyond (genetics, genomics, other 'omics' technologies)
12:30 - 14:00	Lunch
14:00 - 15:15	02 - KLKs in malignant diseases (biomarkers and pathophysiology)
15:15 - 15:45	Coffee break
15:45 - 16:15	02 - KLKs in malignant diseases (biomarkers and pathophysiology)
16:15 - 17:30	Poster session
17:30 - 19:30	Welcome cocktail

Thursday
26 September 2019

9:15 - 10:30	03 - KLKs in physiology and pathophysiology (physiologic systems and diseases)
10:30 - 11:00	Coffee break
11:00 - 12:15	03 - KLKs in physiology and pathophysiology (physiologic systems and diseases)
12:15 - 13:30	Lunch and Poster session
13:30 - 15:00	04 - KLKs in tissue and cell signaling
15:00 - 15:30	Coffee break
15:30 - 16:45	05 - Therapeutic potential of KLKs (& Drug design)
19:00 - 22:00	Gala dinner

Friday
27 September 2019

9:15 - 10:30	06 - Structural and functional aspects of Kallikreins, their substrates and inhibitors
10:30 - 11:00	Coffee break
11:00 - 11:45	07 - KLKs in proteolytic (activation) networks
11:45 - 13:00	Lunch
13:00 - 14:30	08 - HENNER GRAEFF Foundation & E.K. Frey - Werle Foundation session
14:30 - 14:45	ISK2019 Young researchers awards
14:45 - 15:15	Closing lecture - Eleftherios Diamandis - "Kallikrein memoirs and future predictions"
15:15 - 15:20	Closing

LIST OF INVITED SPEAKERS

Chahrazade El Amri (France)	*"Pharmacological modulators of kallikrein-related peptidases: conception, characterisation and therapeutical applications in inflammation and neurodegeneration"*
Jyotsna Batra (Australia)	*"A functional PSA prostate cancer susceptibility genetic variant confers both reduced and aggressive cancer risk"*
Yves Courty (France)	*"Differential roles of KLKs in influenza virus infection"*
Eleftherios P. Diamandis (Canada)	*"Kallikrein memoirs and future predictions"*
Peter Goettig (Austria)	*"The dynamic structure of KLK8 in activity and inhibition"*
Jonathan Harris (Australia)	*"Design and recombinant production of a bispecific inhibitor of KLK5 and KLK7"*
Morley Hollenberg (Canada)	*"Proteinase-mediated signalling in the inflammatory tissue microenvironment: KLKs, proteinase-activated receptors (PARs) and more"*
Tomasz Kantyka (Norway)	*"Processing of pro-MMPs by KLK14 - a new level of extracellular matrix proteolysis"*
Adriana Krizova (Canada)	*"Distinct KLK3 expression can aid pathologists in differential diagnosis of benign structures vs. prostatic carcinoma"*
Daniela Loessner (UK)	*"Kallikrein-related peptidases are key elements in the tumour microenvironment"*
Andreas Scorilas (Greece)	*"Identification of novel Kallikrein gene isoforms and their targeted non conding RNAs. A new area of novel tumor biomarkers"*
Georgia Sotiropoulou (Greece)	*"Revealing unsuspected roles of KLKs in skin pathologies"*
Tangsheng Yi (USA)	*"KLK5 in airway inflammation and epithelial barrier dysregulation"*
George Yousef (Canada)	*"In silico navigation of kallikrein gene expression in different malignancies and their potential clinical utility"*

Wednesday 25 September 2019

10:00 - 10:30	**Opening** – Radislav Sedláček (Czech Republic)
10:30 - 12:30	**01 - KLK genetics and beyond (genetics, genomics, other 'omics' technologies)**
	Session chair: Hans Lilja
10:30 - 11:00	Andreas Scorilas (Greece) - "Identification of novel Kallikrein gene isoforms and their targeted non conding RNAs. A new area of novel tumor biomarkers"
11:00 - 11:15	Beatriz Pérez-González (Spain) - "Epigenetic regulation of the Kallikrein family"
11:15 - 11:45	*Coffee break*
11:45 - 12:15	Joytsna Batra (Australia) - "A functional PSA prostate cancer susceptibility genetic variant confers both reduced and aggressive cancer risk"
12:15 - 12:30	Hannu Koistinen (Finland) - "KLK transcript levels in prostate cancer"
12:30 - 14:00	*Lunch*
14:00 - 15:15	**02/1st part - KLKs in malignant diseases (biomarkers and pathophysiology)**
	Session chair: Judith Clements
14:00 - 14:30	George Yousef (Canada) - "In silico navigation of kallikrein gene expression in different malignancies and their potential clinical utility"
14:30 - 14:45	Adriana Krizova (Canada) - "Distinct KLK3 expression can aid pathologists in differential diagnosis of benign structures vs. prostatic carcinoma"
14:45 - 15:00	Maria Brattsand (Sweden) - "KLK4 and Prostate Cancer"
15:00 - 15:15	Annie Ren (Canada) - "Individualizing ovarian cancer treatment with KLKs as novel personalized biomarkers of therapeutic response and relapse"
15:15 - 15:45	*Coffee break*
15:45 - 16:15	**02/2nd part - KLKs in malignant diseases (biomarkers and pathophysiology)**
	Session chair: George Yousef
15:45 - 16:00	Weiwei Gong (Germany) - "Clinical relevance of kallikrein-related peptidase 5 and 7 mRNA expression levels in tumor tissue of advanced high-grade serous ovarian cancer patients (FIGO III/IV)"
16:00 - 16:15	Julia Dorn (Germany) - "High levels of KLK7 protein expression are related to a favorable prognosis in triple-negative breast cancer patients"
16:15 - 17:30	**Poster session**
17:30 - 19:30	**Welcome cocktail**

Thursday 26 September 2019

9:15 - 10:30	**03/1st part - KLKs in physiology and pathophysiology (physiologic systems and diseases)** Session chair: Maria Brattsand
9:15 - 9:45	Georgia Sotiropoulou (Greece) - "Revealing unsuspected roles of KLKs in skin pathologies"
9:45 - 10:00	Evgeniya Petrova (France) - "Spink5 conditional knock-out mice represent a viable model of Netherton syndrome allowing further understanding of skin and immune abnormalities"
10:00 - 10:30	Alain Hovnanian (France) - "Epidermis-specific Kallikrein 14 overexpression in transgenic mice leads to major hair shaft defects, desmoglein 3 and 4 degradation and skin inflammation"
10:30 - 11:00	*Coffee break*
11:00 - 12:15	**03/2nd part - KLKs in physiology and pathophysiology (physiologic systems and diseases)** Session chair: Alain Hovnanian
11:00 - 11:30	Yves Courty (France) - "Differential roles of KLKs in influenza virus infection"
11:30 - 11:45	Woodys Lenga Ma Bonda (France) - "Role of the kallikrein-related peptidase 5 in the bronchial epithelial remodeling associated with chronic obstructive pulmonary disease and lung cancer"
11:45 - 12:15	Tangsheng Yi (United States) - "KLK5 in airway inflammation and epithelial barrier dysregulation"
12:15 - 13:30	*Lunch and Poster Session*
13:30 - 15:00	**04 - KLKs in tissue and cell signaling** Session chair: Georgia Sotiropoulou
13:30 - 14:00	Morley Hollenberg (Canada) - "Proteinase-mediated signalling in the inflammatory tissue microenvironment: KLKs, proteinase-activated receptors (PARs) and more"
14:00 - 14:15	Oliver Goldhardt (Germany) - "Kallikrein-related peptidase 6 in cerebrospinal fluid of patients with alzheimer's disease is associated with CSF-TAU, FDG and AMYLOID-PET"
14:15 - 14:30	Klaudia Brix (Germany)- "Kallikreins and cysteine cathepsins act at opposite poles of thyroid epithelial cells"
14:30 - 14:45	Darian Williams (United States) - "The role of mechanosensitive KLK10 in endothelial biology and atherosclerosis"
14:45 - 15:00	Michael Jeltsch (Finland) - "KLK3 activates VEGF-C and VEGF-D"
15:00 - 15:30	*Coffee break*
15:30 - 16:45	**05 - Therapeutic potential of KLKs (& Drug design)** Session chair: Morley Hollenberg
15:30 - 16:00	Jonathan Harris (Australia) - "Design and recombinant production of a bispecific inhibitor of KLK5 and KLK7"
16:00 - 16:15	Caitlin Di Paolo (Canada) - "Novel putative kallikrein-7 inhibiting compounds identified through a fluorescence-based enzyme kinetic screen of a targeted library"
16:15 - 16:45	Chahrazade El Amri (France) - "Pharmacological modulators of kallikrein-related peptidases: Conception, characterisation and therapeutical applications in inflammation and neurodegeneration"
19:00 - 22:00	**Gala dinner (limited to participants registered for the dinner)**

Friday 27 September 2019

9:15 - 10:30	**06 - Structural and functional aspects of Kallikreins, their substrates and inhibitors**
	Session chair: Jonathan M. Harris
9:15 - 9:45	Peter Goettig (Austria) -"The dynamic structure of KLK8 in activity and inhibition"
9:45 - 10:15	Hans Lilja (United States) - "Elucidating the effects of chymotrypsin-like and trypsin-like catalytic activity by prostate specific antigen and human kallikrein 2 on prostate tissue microenvironment, tumorigenesis and blood release"
10:15 - 10:30	Niv Papo (Israel) - "Mapping protein selectivity landscapes using multi-target selective screening and next-generation sequencing of combinatorial libraries"
10:30 - 11:00	*Coffee break*
11:00 - 11:45	**07 - KLKs in proteolytic (activation) networks**
	Session chair: Peter Goettig
11:00 - 11:15	Tomasz Kantyka (Norway) - "Processing of pro-MMPs by KLK14 - a new level of extracellular matrix proteolysis"
11:15 - 11:30	Katharina Käfinger (Germany) - "KLK4-mediated cleavage of CXCR3-ligands as an immune evasion mechanism in ovarian cancer"
11:30 - 11:45	Leran Zhang (United Kingdom) - "Unlocking the KLK activome in drug-resistant cancer: imaging, biomarkers and target validation using novel activity probes"
11:45 - 13:00	*Lunch*
13:00 - 14:30	**08 - HENNER GRAEFF Foundation & E.K. Frey - Werle Foundation session**
13:00 - 13:10	Viktor Magdolen (Germany) - History of HENNER GRAEFF Foundation & E.K. Frey - Werle Foundation of the Henning L. Voight Family
13:10 - 13:20	Judith Clements (Australia) - Laudation for the 2019 Joint Frey-Werle Foundation and Henner Graeff Foundation Promotion Prize Awardee
13:20 - 13:50	Daniela Loessner (United Kingdom) - Promotion Prize Awardee - "Kallikrein-related peptidases are key elements in the tumour microenvironment"
13:50 - 14:10	Henner Graeff Foundation Young Investigator Award - Amiram Sananes (Israel) - "Combinatorial Engineering of APPI Variant with Improved Proteolytic Resistance and KLK6 Selectivity for Cancer Imaging and Therapy"
14:10 - 14:30	E.K. Frey - Werle Foundation - Young Investigator Award - Srilakshmi Srinivasan (Australia) - "Prostate cancer risk associated Single Nucleotide Polymorphism affects PSA glycosylation and its function"
14:30 - 15:20	**Closing**
14:30 - 14:45	ISK2019 Young researchers awards (Radislav Sedláček, Czech Republic)
14:45 - 15:15	Closing lecture - Eleftherios Diamandis (Canada) - "Kallikrein memoirs and future predictions"
15:15 - 15:20	Closing - Radislav Sedláček

SESSION 01
KLK genetics and beyond (genetics, genomics, other 'omics' technologies)

Wednesday 25 September 2019

Session chair: Hans Lilja

Andreas Scorilas (Greece)
"Identification of novel Kallikrein gene isoforms and their targeted non conding RNAs. A new area of novel tumor biomarkers"

Beatriz Pérez-González (Spain)
"Epigenetic regulation of the Kallikrein family"

Joytsna Batra (Australia)
"A functional PSA prostate cancer susceptibility genetic variant confers both reduced and aggressive cancer risk"

Hannu Koistinen (Finland)
"KLK transcript levels in prostate cancer"

Identification of novel Kallikrein gene isoforms and their targeted non conding RNAs. A new area of novel tumor biomarkers.

Andreas Scorilas[1]

1. Department of Biochemistry and Molecular Biology, National and Kapodistrian University of Athens, Greece

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Tissue Kallikrein and Kallikrein-Related Peptidase Genes (KLKs) share significant sequence and structural similarity, as well as conserved exon/intron structure. Multiple alternative transcripts have been reported for most human KLKs, and many encode different protein isoforms, some of which appear to be specifically expressed in different types of cancer tissues. The family of non-coding RNAs (ncRNAs) includes several recently identified RNA, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) implicated mostly in gene expression regulation at the transcriptional and post-transcriptional levels. The ability of ncRNAs to regulate gene expression has led to intense investigation of their functional role during cancer development and progression, as well as to elucidate their clinical value in patients' prognosis. Next-generation sequencing (NGS) technology has showed unprecedented impact on the analysis of human cancer cell genome, offering high-throughput DNA and RNA sequencing.Our research group has developed targeted NGS methodologies to unveil novel alternative transcripts of the human KLK gene family. The resulting NGS data were analysed with bioinformatics algorithms that we developed for this purpose. As a result, a total of more than 80 novel transcripts for the targeted KLK genes were identified. Many novel KLK transcripts are predicted to encode new protein isoforms, whereas others are nonsense-mediated mRNA decay (NMD) candidates. We have also developed a highly sensitive quantitative methodology in order to evaluate the clinical significance of KLK-targeting ncRNAs in breast, prostate, bladder and colorectal malignancies. Our results reveal that there are many ncRNA molecules which targets KLKs transcripts and they can provide significant information regarding the prediction of disease outcome and to display important discriminatory capacity between malignant and non cancer samples. The role of KLK-related ncRNAs as new generation of multifactorial biomarkers in different type of cancers beginning to unravel.Acknowledgement: Part of this study was supported by Hellenic Society of Medical Oncology (HeSMO)

Epigenetic regulation of the Kallikrein family

Beatriz Pérez-González[1], Joan Gil[1], Núria Villalmanzo[1], Mireia Jordà[1]

1. Program of Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), Badalona, Spain

E-mail of the presenting author: bperez@igtp.cat

Kallikreins (KLKs) represent one of the biggest genetic cluster in the human genome (around 265 kb) but the regulation at a genomic level is still poorly understood. Previous results from our laboratory showed that some KLKs display aberrant DNA methylation in thyroid cancer. Moreover, the analysis of their expression revealed differential and specific expression profiles associated with the main driver mutations in thyroid cancer, BRAF or RAS, outlining three different domains of expression. Importantly, these domains were maintained in other types of cancer, although displaying different profiles. Therefore, we hypothesize that the KLK cluster is divided in three expression domains that are regionally coregulated through cis interactions between KLK promoters from different domains and from the same domain and, additionally, distal regulatory elements. First, we identified a region located within KLK cluster that is predicted by chromatin state available data from ENCODE to be a strong enhancer only in those cell lines that express KLKs. Performing luciferase reporter assays, we confirmed its enhancer activity, which was higher in cells expressing KLKs. Moreover, we were able to upregulate the expression of KLKs when activating the enhancer using the CRISPR-SAM technique. Preliminary results checking the DNA methylation levels of the enhancer seem to point out that this epigenetic mechanism is playing a minor role in the regulation of this cluster. Although further analyses are currently being performed, such as UMI-4C, to better understand the underlying mechanism of regulation of the KLK family, these results suggest a cross-talk of KLK promoters via a shared enhancer.

A functional PSA prostate cancer susceptibility genetic variant confers both reduced and aggressive cancer risk

Srilakshmi Srinivasan[1,2], Thomas Kryza[1,2], Nathalie Bock[1,2], Brian Tse[3], Kamil Sokolowski[3], Hans Lilja[4], The Practical Consortium[5], The Australian Prostate Cancer BioResource[2], Judith Clements[1,2], Jyotsna Batra[1,2]

1. Cancer Program, Institute of Health and Biomedical Innovation and School of Biomedical Sciences, Queensland University of Technology, Brisbane, Queensland, Australia
2. Translational Research Institute, Translational Research Institute, Woolloongabba, Brisbane, Australia
3. Preclinical Imaging Facility, Translational Research Institute, Woolloongabba, Brisbane, Australia
4. Departments of Laboratory Medicine, Surgery (Urology Service) and Medicine (Genitourinary Oncology), Memorial Sloan Kettering Cancer Center, New York, USA
5. Centre for Cancer Genetic Epidemiology, Cambridge, UK

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Prostate cancer susceptibility is influenced by common variants at multiple loci, however, the mechanisms by which these variants influence prostate cancer risk remain largely unknown. The rs17632542 single nucleotide polymorphism (SNP) in exon 4 of the kallikrein-related peptidase 3 (KLK3) gene encoding prostate-specific antigen (PSA) was previously questioned for its association with prostate cancer due to its association with PSA levels as well. We aimed to verify that this SNP plays a functional role in mediating prostate cancer risk and progression. The non-synonymous rs17632542 SNP (c.536T>C), is associated with prostate cancer risk, Odds Ratio (OR=0.74, 95% CI 0.72-0.76, P= 6.69 x 10-81 and survival, Hazards ratio (HR)=1.33, 95% CI=1.24-1.45, P<0.001 in our large cohort of 79,194 prostate cancer cases and 61,112 disease-free controls. The rs17632542 SNP leads to amino acid change 'Ile' to 'Thr' at position 161, which lowers the proteolytic activity of PSA towards extracellular matrix proteins and diminishes the proliferation and migration of prostate cancer cells. In addition, the 'Thr' PSA protein variant formed small localised tumours but aggressive metastasis in our in vivo models indicating that presence of the SNP is deleterious in patients with aggressive disease. The minor 'C' allele leads to lower levels of serum PSA-inhibitor complexes and is associated with higher free PSA levels suggesting that the SNP could potentially lead to detection bias affecting the clinical management of the disease. These findings suggest that accounting for the rs17632542 SNP effects may reduce the inaccuracies of prostate cancer diagnosis based on total PSA levels alone.

KLK transcript levels in prostate cancer

Hannu Koistinen[1], Timo-Pekka Lehto[1], Adrian Malen[1], Carolin Stürenberg[1], Andrew Erickson[1], Antti Rannikko[1], Tuomas Mirtti[1]

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The prostate produces several kallikrein-related peptidases, the most abundant ones being KLK3 (prostate-specific antigen, PSA) and KLK2, which have been used as prostate cancer markers. However, to date no widely-accepted biomarkers of clinically relevant prostate cancer exist. The greatest unmet clinical need is for prognostic markers for patients with Gleason score (GS) 7 or 8 cancers, as such patients cannot be risk-stratified with existing histological and clinical information with reasonable accuracy. We studied mRNA levels of all 15 human KLKs in prostate cancer, especially whether the levels are able to distinguish aggressive cancers from non-aggressive ones in a subgroup of GS7 and 8 cancers. The expression of proteinase-activated receptors (PARs) was also addressed.

Based on the clinically relevant end-points of disease specific death, metastatic progression or implementation of chemotherapy during the follow-up of 1 to 22 years, 75 patients with GS7 or 8 prostate cancers were selected to present aggressive cases. Patients (n=85) with similar clinical characteristics and GS, but no clinically relevant end-point, were assigned as controls. Also samples from other groups, like patients with benign prostatic diseases, were included. mRNA was extracted from FFPE blocks and transcript levels were detected using NanoString technology and custom code set panel containing KLKs and a panel of housekeeping genes, used for normalization.

KLK3, -2, -4, -11, -15, -12, -14 and -10 (in decreasing order) were significantly expressed in GS7/8 prostate cancers. Although the expression levels of several KLKs were changed in cancer, as compared to benign prostate, the levels were mostly similar in aggressive cancer and control groups. Among the PARs, especially PAR-1 and PAR-2 were expressed in prostate, and again no major differences were found between the aggressive cancer and control groups. In conclusion, assessing the transcript levels of individual KLKs in prostate tissue may not be clinically useful in distinguishing aggressive and non-aggressive cancers in the subgroup of GS7/8 cancers, i.e., the group with the greatest unmet clinical need for novel markers.

SESSION 02
KLKs in malignant diseases (biomarkers and pathophysiology)

Wednesday 25 September 2019

1st part
Session chair: Judith Clements

George Yousef (Canada)
"In silico navigation of kallikrein gene expression in different malignancies and their potential clinical utility"

Adriana Krizova (Canada)
"Distinct KLK3 expression can aid pathologists in differential diagnosis of benign structures vs. prostatic carcinoma"

Maria Brattsand (Sweden)
"KLK4 and Prostate Cancer"

Annie Ren (Canada)
"Individualizing ovarian cancer treatment with KLKs as novel personalized biomarkers of therapeutic response and relapse"

2nd part
Session chair: George Yousef

Weiwei Gong (Germany)
"Clinical relevance of kallikrein-related peptidase 5 and 7 mRNA expression levels in tumor tissue of advanced high-grade serous ovarian cancer patients (FIGO III/IV)"

Julia Dorn (Germany)
"High levels of KLK7 protein expression are related to a favorable prognosis in triple-negative breast cancer patients"

In silico navigation of kallikrein gene expression in different malignancies and their potential clinical utility

Sicheng Lin[1,4], Sung Sun Kim[1,2], Liping Ou[1], Qiang Ding[1], George Yousef[1,2,3]

1. Department of Pathology and Laboratory Medicine, Li Ka Shing Knowledge Institute, St. Michael's Hospital
2. Department of Laboratory Medicine and Pathobiology, University of Toronto
3. Department of Paediatric Laboratory Medicine, Hospital for Sick Children
4. Western University

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Introduction:
Recently, many biological databases are open to the public, and these data are important sources of well-organized and highly curated data. These databases and their associated analytical tools enable the discovery of biological markers, validate experimental data, and globally analyze specific gene or molecular changes in cancer. Kallikreins (KLKs) are a family of 15 genes that has known to be related to cancer.
Aim: We interrogated publicly accessible database for KLK expression in adult tumors to elucidate their potential contribution to cancer pathogenesis and their possible utility as cancer biomarkers.

Materials and methods:
We used cBioPortal and FireBrowse, which are in-depth data analysis tools that use large numbers of dataset from The Cancer Genome Atlas (TCGA). All 15 KLKs in 37 different cancer cohorts were analyzed. Each KLK was analyzed for mRNA expression level, chromosomal aberrations, and mutations. Kaplan-Meier plot of each KLK in a specific cancer type was obtained by using graphical tools.

Results:
30 cancer types had dysregulated KLK expressions. Nearly half of these cancers had elevated expression of most KLKs, and KLK expression was downregulated compared to normal in one-third of the cancers. For example, KLK4 significantly decreased in thyroid carcinoma compared to normal while upregulated in breast carcinoma. From a mutational perspective, on average KLKs mutation rate was < 5%, mostly variants of unknown significance, indicating that mutations play a minor role in KLK dysregulation in cancer. Individual KLKs were found to have higher mutational rate for specific cancers, like KLK3 in cutaneous melanoma. Chromosomal aberrations were also identified for KLKs, for example, KLK3 showed amplification in uterine carcinosarcoma. Some KLK gene expressions were associated with prognosis. For example, higher expression of KLK4 is associated with worse prognosis in esophageal carcinoma. ($p = 0.0323$)

Conclusion:
Using public databases, we identified KLK genes that are dysregulated in cancer and can also have prognostic utility to predict cancer behaviour. Further experimental validation on independent dataset are needed to confirm these results.

Distinct KLK3 expression can aid pathologists in differential diagnosis of benign structures vs. prostatic carcinoma

Adriana Krizova[1,2], Kim Sungsun[1], Arsani Yousef[1], Zaki John[1]

1. St Michaels Hospital
2. University of Toronto

E-mail of the presenting author: krizovaadr@smh.ca

Introduction:
KLK3 (PSA, prostate specific antigen) is a protease of seminal plasma that aids in liquefaction of the seminal coagulum. Serum PSA is used for screening and follow-up of prostate cancer. KLK3 is expressed in normal prostate tissue and prostate cancer. Previous studies have shown that higher grade prostate cancer shows weaker expression of KLK3.

Aim:
We assessed KLK3 expression in normal structures (ejaculatory system, periurethral, central and peripheral zones), benign changes (basal cell hyperplasia, atrophy), precancerous lesion (high-grade intraepithelial neoplasia, HGPIN) and various grades of acinar adenocarcinoma.

Methods:
Radical prostatectomy specimens were selected. Monoclonal KLK3 antibody was used for staining. Staining interpretation was done by a pathologist.

Results:
KLK3 expression was found in the cytoplasm of peripheral and central zone acini. There was no staining in the ejaculatory system, urothelium, atrophic glands and basal cell hyperplasia. There was variation in intensity of KLK3 cytoplasmic staining in HGPIN. In the acinar adenocarcinoma, Gleason grade 3 glands revealed strong diffuse positive cytoplasmic staining with frequent luminal accentuation. Among the Gleason grade 4 glands, cribriform/fused glands showed moderate to strong diffuse staining pattern, whereas abortive glands often revealed weak to moderate staining with luminal accentuation in the reminiscent lumens. In the Gleason grade 5 glands, solid sheets or glands with central necrosis showed weak positivity and single malignant cells revealed rare reactivity for KLK3.

Conclusion:
Given that KLK3 is a standard immunohistochemical stain in many pathology laboratories, distinct KLK3 cytoplasmic expression can aid in the differential diagnosis of benign structures vs malignancy especially in limited diagnostic material (atrophy/ejaculatory system/ basal cell hyperplasia (KLK3-) vs. Gleason grade 3 adenocarcinoma (KLK+); ejaculatory ducts/transitional epithelium (KLK3-) vs. HGPIN (KLK3+)).

KLK4 and Prostate Cancer

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Prostate cancer (PC) is a major global medical problem, and in Sweden it is the most frequently diagnosed tumor disease with about 10,000 new cases per year. Growth of normal prostate tissue as well as prostate adenocarcinoma is regulated by androgens, therefore surgical or medical reduction of androgens (ADT) is the standardized treatment for advanced PC patients. After first remission, tumors will return and is then known as CRPC (Castration-resistant PC). When this happens, the tumor is no longer responding to castration treatment, and all cases of CRPC have a fatal outcome, which clearly points to the need for new therapies for CRPC.

PC is a very multifaceted disease, and it is not possible to determine today whether the tumor found will develop into an aggressive form or not. The mechanisms behind PC metastasis and castration resistant tumor growth are largely unknown and identification of molecular pathways involved in this has the potential to improve PC prognosis.

Analysis of an Illumina expression array showed very high levels of KLK4 in normal prostate and primary tumor tissues. In bone metastasis, the average levels were reduced to half but still the levels were much higher than in normal bone or bone metastasis from other tumor diseases. However, no KLK4 protein was identified in data from peptide analysis which was quite unexpected due to the high transcript levels found. RACE-PCR followed by DNA sequencing showed that the KLK4 transcripts present all lacked the 5' end coding for the signal sequence, and a vast majority of the transcripts started downstream of the pro-peptide. Attempts to express the cDNA in PC3 cells failed in detecting any KLK4 protein in the cells although high levels of transcripts present. In order to elucidate the function, KLK4 transcripts were knocked out in 22Rv1, a prostate cancer cell line with high ectopic mRNA levels, using the shSMARTer Lentivirus system. The effects were analyzed using mRNA and miRNA expression arrays.

Individualizing ovarian cancer treatment with KLKs as novel personalized biomarkers of therapeutic response and relapse

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Ovarian cancer (OC) remains hard to treat due to a high risk of relapse and chemoresistance. With new therapies on the horizon, there is an unmet need for biomarkers that catch early relapse and pinpoint the best time for starting or changing therapy. The classical OC biomarker, CA125, is controversial for monitoring as it is elevated in only 70% of patients with imaging confirmed relapse and not elevated at all in 20% of advanced disease.

Owing to vast tumour heterogeneity, we propose that each tumour secretes a distinct array of proteins into the blood, which if quantified could serve as personalized tumour markers. KLKs, being implicated in OC tumorigenesis, are expected to be amongst the tumour protein signature. Future screening against a panel of personalized markers could identify the best biomarkers for monitoring each patient in order to guide individualized treatment strategies.

To discover personalized OC markers, we used a new multiplex proteomics technology (Proseek® panels, Olink, Sweden), which simultaneously performs 1000+ ELISA-based proximity extension assays, to measure 1104 proteins [including five KLKs implicated in OC (KLK 6, 8, 10, 11 and 14)] in sera of 30 OC patients collected pre-operation, 3 weeks post-op, 5 months and 11 months post-op/at relapse. Non-cancer and healthy controls (N=21) were used. We hypothesized that proteins that significantly decreased post-op and increased upon relapse may reflect tumour burden. We identified a panel of 22 proteins (including five KLKs) that is predictive of recurrence even in CA125-uninformative patients (28% of cohort). KLK10 and 14 were each informative of relapse for one patient, KLK6 and 11 were each informative for two patients, and KLK8 was informative for three patients. The rise in KLK levels upon relapse in these patients exceeded that of CA125, hinting KLKs as more robust biomarkers in these cases.

With future validation, a panel of personalized markers including KLKs can be used to sensitively track OC tumours in each patient, ultimately playing key roles in customizing patient management and treatment. Our panel also helps identify patients who may benefit from new KLK-targeted therapies. The novel concept of our study can be adapted for various cancers, serving as a small but paramount step towards introducing KLKs to the precision medicine scene.

Clinical relevance of kallikrein-related peptidase 5 and 7 mRNA expression levels in tumor tissue of advanced high-grade serous ovarian cancer patients (FIGO III/IV)

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Most members of the kallikrein-related peptidase (KLK) family have been reported to be dysregulated in ovarian cancer. Some of these KLKs may represent biomarkers for diagnosis, prognosis, and response of treatment. In the present study, we determined KLK5 and KLK7 mRNA expression levels in tumor tissue of a homogeneous patient cohort encompassing 139 patients afflicted with advanced high-grade serous ovarian cancer and related the expression levels with established clinical factors and patients‘ outcome.

High correlations were observed between KLK5/KLK7 mRNA expression (r_s=0.568, p<0.001) and KLK5/KLK7 protein expression (r_s=0.805, p<0.001), respectively. A significant association between KLK5 mRNA expression and residual tumor mass (p=0.041) was observed. There were no further significant associations between KLK5 or KLK7 mRNA expression levels and established clinical factors.

In univariate Cox regression analysis, elevated KLK5 mRNA levels were significantly linked with shorter progression-free survival (PFS; hazard ratio [HR]=1.60, p=0.047). Regarding overall survival (OS), no significant correlation with KLK5 mRNA expression levels was observed. High KLK7 mRNA expression levels were shown to be markedly correlated with poor PFS (HR=1.75, p=0.025) and showed a trend towards significance in case of OS (HR=1.66, p=0.055).

In multivariable analysis, KLK5 mRNA expression did not prove to be statistically significant, however, showed a tendency with regard to statistical significance in case of PFS (HR=1.53, p=0.095). Elevated KLK7 mRNA values turned out to be significantly linked with both shortened PFS (HR=2.19, p=0.007) and OS (HR=1.94, p=0.032).
In conclusion, in univariate analysis, elevated KLK5 mRNA expression is significantly related with shortened PFS in advanced high-grade serous ovarian cancer. Elevated KLK7 mRNA expression was identified as an independent unfavorable prognostic marker for both PFS and OS in this major subtype of ovarian cancer. KLK5 and KLK7 may thus represent novel targets for therapy of ovarian cancer.

High levels of KLK7 protein expression are related to a favorable prognosis in triple-negative breast cancer patients

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In normal physiology, KLK7 - in concert with other members of the kallikrein-related peptidase family - is mainly involved in skin desquamation and keratinization processes. Expression of KLK7 was shown to be upregulated in various tumor types including colon or ovarian cancer but downregulated in breast cancer. While high levels of tumor-tissue KLK7 correlate to a shorter patient survival time in colon or ovarian cancer, there are contradictory reports in breast cancer whether KLK7 represents a un- or favorable prognostic biomarker.

In the present study, we examined the prognostic value of KLK7 protein expression levels in triple-negative breast cancer (TNBC), determined by immunohistochemistry (IHC). A cohort encompassing 133 patient tumor samples afflicted with TNBC, present on tissue microarrays, was analyzed. For quantification, an automated digital IHC image analysis algorithm was applied. In both Kaplan-Meier and univariate Cox analyses, elevated KLK7 protein levels were significantly linked with prolonged overall survival (OS). In multivariate Cox analysis, addition of KLK7 immunoreactive scores to the base model (including the clinical parameters age, tumor size, and nodal status) demonstrated that - in addition to the nodal status of the patients - KLK7 protein expression remained as an independent predictive marker for OS.

These results strongly indicate that KLK7 is a favorable prognostic biomarker in triple-negative breast cancer.

SESSION 03
KLKs in physiology and pathophysiology (physiologic systems and diseases)

Thursday 26 September 2019

1st part
Session chair: Maria Brattsand

Georgia Sotiropoulou (Greece)
"Revealing unsuspected roles of KLKs in skin pathologies"

Evgeniya Petrova (France)
"Spink5 conditional knock-out mice represent a viable model of Netherton syndrome allowing further understanding of skin and immune abnormalities"

Alain Hovnanian (France)
"Epidermis-specific Kallikrein 14 overexpression in transgenic mice leads to major hair shaft defects, desmoglein 3 and 4 degradation and skin inflammation"

2nd part
Session chair: Alain Hovnanian

Yves Courty (France)
"Differential roles of KLKs in influenza virus infection"

Woodys Lenga Ma Bonda (France)
"Role of the kallikrein-related peptidase 5 in the bronchial epithelial remodeling associated with chronic obstructive pulmonary disease and lung cancer"

Tangsheng Yi (United States)
"KLK5 in airway inflammation and epithelial barrier dysregulation"

Spink5 conditional knock-out mice represent a viable model of Netherton syndrome allowing further understanding of skin and immune abnormalities

Evgeniya Petrova[1], Florent Leturcq[1], Claire Barbieux[1], Alain Hovnanian[1,2,3]

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Netherton syndrome (NS) is a severe autosomal recessive skin disorder caused by loss-of-function mutations in SPINK5. The disease is characterized by severe skin desquamation and hair shaft defect, which are accompanied by inflammation and allergic reactions. SPINK5 encodes the serine protease inhibitor LEKTI, which regulates epidermal protease activity during the desquamation process in normal epidermis. Absence of LEKTI expression leads to disturbed epidermal protease activity, resulting in stratum corneum detachment from the granular layer, loss of skin barrier function and Par2 activation resulting in skin inflammation.

Constitutive Spink5-deficient mice reproduce the NS phenotype, but die from dehydration within few hours after birth due to severe skin barrier defect, thus preventing further study of disease progression. To circumvent this problem, we generated a conditional Spink5 knock-out mouse model (Spink5 cKO) by crossing mice carrying LoxP site-containing Spink5 alleles with the K14-Cre-ERT2 transgenic line, thus enabling tamoxifen-inducible Cre-mediated recombination in basal layer keratinocytes. Induction of Spink5 deletion in the epidermis of young adult mice resulted in the development of clinical and biological features of NS within two weeks after tamoxifen injection.

Spink5 cKO mice showed red, scaly and crusty skin with alopecic areas secondary to scratching behavior. Increased transepidermal water loss indicated a skin barrier defect, which correlated with the severity of NS clinical features. Tissue-level analyses revealed thickening of the epidermis, stratum corneum detachment, altered expression of epidermal differentiation and proliferation markers, along with increased protease activity in the skin. In addition to skin abnormalities, Spink5 cKO mice displayed features of systemic inflammation response as evidenced by enlarged spleen and lymph nodes, thymic atrophy as wells as increased number of skin-infiltrating immune cells.

Epidermis-specific Spink5 ablation reproduces a viable NS phenotype, which makes it a convenient model for investigating skin and immune system anomalies in adult animals and for in vivo testing of new therapeutic approaches.

Epidermis-specific Kallikrein 14 overexpression in transgenic mice leads to major hair shaft defects, desmoglein 3 and 4 degradation and skin inflammation

Olivier Gouin[1,2], Claire Barbieux[1,2], Florent Leturcq[1,2], Mathilde Bonnet des Claustres[1,2], Evgeniya Petrova[1,2], Alain Hovnanian[1,2,3]

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Netherthon syndrome (NS) is a rare autosomal recessive skin disease caused by loss-of-function mutations in SPINK5 encoding LEKTI. LEKTI deficiency results in unopposed activity of epidermal kallikrein-related peptidases (KLKs), mainly KLK5, KLK7 and KLK14. While the function of KLK5 and KLK7 has been previously studied, the role of KLK14 in skin homeostasis and its contribution to NS pathogenesis remain unknown. To gain insight into KLK14 function in vivo, we generated a transgenic murine model overexpressing human KLK14 (TghKLK14) in the upper epidermal layers. TghKLK14 mice showed increased caseinolytic activity in the granular layers and in hair follicles. hKLK14 overactivity led to the absence of hair growth and hyperplastic hair follicles with disorganized hair shafts. Ultrastructural analysis revealed cell separation in the hair cortex. Dsg2 staining was increased while Dsg3 and Dsg4 were markedly reduced. In vitro studies showed that hKLK14 directly cleaves rhDSG3 and rhDSG4, suggesting that their degradation contributes to hair abnormalities. The epidermis of TghKLK14 mice was hyperproliferative and their skin showed an inflammatory signature involving innate immunity. This in vivo study identifies KLK14 as an important contributor to hair abnormalities and skin inflammation seen in NS.

Differential roles of KLKs in influenza virus infection

Yves Courty[1]

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Influenza A viruses (IAV) cause acute infection of the respiratory tract that affects millions of people during seasonal outbreaks every year. The HA (hemagglutinin) of influenza virus must be activated by proteolysis before the virus can become infectious. Previous studies have indicated that HA cleavage is driven by membrane-bound or extracellular serine proteases. We examined the potential activating role of KLK1, KLK5, KLK8, KLK13 and KLK14. Recombinant HAs from IAV of the H1N1 subtype were cleaved by KLK1, KLK5 and KLK14 whereas only KLK5 and KLK14 were able to process HAs from viruses belonging to the H3N2 subtype. Pretreament of noninfectious virions with these KLKs revealed a more restraint pattern of activation with a preference of KLK14 for activation of H1N1 virus and of KLK5 for H3N2 virus. The five KLKs were quantified in tracheal aspirates from patients in intensive care as a result or not of seasonal influenza. Secretion of KLK5 was found selectively enhanced in patients with influenza infection. Moreover, we showed that pretreatment of airway secretions with a KLK5-selective inhibitor significantly reduced the activation of H3N2 virions.

Host cell proteases are also involved in influenza pathogenesis and we assessed the role of KLK1 and KLK14 in the antiviral response using mice models. Infection of mice with IAV (H3N2 subtype) enhanced the concentration of the two KLKs in the bronchoalveolar fluid (BAL). During the early period after infection, the viral load within BAL fluid was significantly lower in WT mice than in KLK1-deficient mice. This indicated that KLK1 plays an early antiviral role. We found that mKLK1 limited the virus-induced apoptosis of alveolar macrophages early in infection. Mice producing KLK1 also showed a transient increase in natural killer cells in the BAL fluid during this early period. Mice lacking KLK14 had a poorer clinical outcome after influenza A virus infection than WT littermates indicating that KLK14 plays a protective role during IAV infection. Indeed, KLK14-deficiency limits macrophage influx in lung of mice. These studies revealed a dual role of pulmonary kallikreins in influenza pathogenesis: a contribution to the replication and dissemination of viruses (KLK5) and protective antiviral actions via the regulation of recruitment and survival of immune cells (KLK1 & 14).

Role of the kallikrein-related peptidase 5 in the bronchial epithelial remodeling associated with chronic obstructive pulmonary disease and lung cancer

Woodys Lenga Ma Bonda[1], Lucie Brisson[2], Laura Regnier[1], Sébastien Roger[3], Yves Courty[1], Sophie Iochmann[1,4], Pascale Reverdiau[1,4]

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According to the WHO, chronic obstructive pulmonary disease (COPD) will be the third leading cause of mortality worldwide by 2030. This inflammatory disorder is associated with lung cancer in the third of patients. COPD is characterized by the thickening of the airway walls and this can lead to the progressive destruction of lung tissue and alveolar walls during exacerbation phases and emphysema. Chronic tobacco smoking, the most important cause of this disease, is responsible for airway damage and induces the production of inflammatory mediators and proteases. We are focusing our study on the human tissue Kallikrein-related peptidase 5 (KLK5), a serine protease that could, as plasmin and metalloproteases (MMP), involved the extracellular matrix (ECM) degradation and tissue remodelling. We demonstrated that KLK5 induces cell detachment of Beas2B epithelial cells and this process is associated with loss of cell adhesion to fibronectin and more weakly to vitronectin and collagen IV. This is explained by the ability of KLK5 to cleave fibronectin rapidly and also some integrins, mainly alpha5beta1 and alpha6beta4. Moreover, during this process cells acquire a mesenchymal phenotype with an increase in cell migration as shown by time lapse videomicroscopy and using modified Boyden chamber assay. In contrast KLK5 has no effect on cell adhesion, migration and invasion of A549 tumour cells. All the data suggest that KLK5 could regulate tissue remodelling and initiate the epithelial-mesenchymal transition to repair bronchial epithelium when impaired due to chronic smoking and inflammatory process.

KLK5 in airway inflammation and epithelial barrier dysregulation

Tangsheng Yi[1], Janet Jackman[1], Juan Zhang[1], David Choy[1], Peipei Lu[1], Hong Li[1], Jiansheng Wu[1], Robert Lazarus[1], Wyne Lee[1], Joseph Arron[1], James Koerber[1], Cary Austin[1], Brian Yaspan[1]

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Clinical studies of T helper 2 (TH2) cytokine neutralizing antibodies in severe asthma have identified a significant subset of patients with low levels of TH2 inflammation who do not benefit from TH2 cytokine neutralizing antibodies. Non-TH2 mechanisms are poorly understood in asthma but represent a significant unmet medical need. Here we utilized an unbiased genome-wide genetic association analysis (GWAS) of moderate-severe asthma patients stratified by high and low TH2 serum biomarkers and identified a novel protective SNP near the kallikrein 5 (KLK5) locus selectively associated with TH2-low asthma. KLK5 is secreted by human bronchial epithelial cells and elevated in severe asthma bronchoalveolar lavage. TH2 cytokines negatively regulate KLK5 activity in human bronchial epithelial cells. Catalytically active KLK5 promotes epithelial permeability, upregulates epithelial chemokine/cytokine expression, and induces neutrophil influx. Taken together, our work identifies KLK5 as a key mediator in regulating lung inflammation and permeability in a TH2 independent manner and suggests that KLK5 inhibitors may represent a novel therapeutic approach for TH2-low asthma.

SESSION 04
KLKs in tissue and cell signaling

Thursday 26 September 2019

Session chair: Georgia Sotiropoulou

Morley Hollenberg (Canada)
"Proteinase-mediated signalling in the inflammatory tissue microenvironment: KLKs, proteinase-activated receptors (PARs) and more"

Oliver Goldhardt (Germany)
"Kallikrein-related peptidase 6 in cerebrospinal fluid of patients with alzheimer's disease is associated with CSF-TAU, FDG and AMYLOID-PET"

Klaudia Brix (Germany)
"Kallikreins and cysteine cathepsins act at opposite poles of thyroid epithelial cells"

Darian Williams (United States)
"The role of mechanosensitive KLK10 in endothelial biology and atherosclerosis"

Michael Jeltsch (Finland)
"KLK3 activates VEGF-C and VEGF-D"

Proteinase-mediated signalling in the inflammatory tissue microenvironment: KLKs, proteinase-activated receptors (PARs) and more

Morley Hollenberg[1]

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Added to their roles in protein processing and degradation, proteinases can now be seen to play 'hormone-like' functions to regulate target tissues by multiple mechanisms ranging from the generation of active polypeptides from pro-hormone precursors to the modulation of signal transduction by regulating cell surface receptors. This 'hormone-like' role of proteinases can have an impact on processes ranging from tissue development to the mechanisms mediating the innate inflammatory immune response and cancer growth/metastasis. Thus, in the setting of tissue inflammation triggered by injury or cancer, the activation and inactivation of proteinases in the extracellular microenvironment can regulate cell function in an autocrine-paracrine hormone-like manner. In this context the kallikrein-related peptidases (KLKs), along with other proteolytic systems that act in a 'cascade manner', like the coagulation and complement cascades, can be seen to have an immense impact on tissue function. This overview will illustrate the importance of extracellular microenvironment proteinase signalling in inflammatory processes and cancer, with attention paid to the potential roles played by KLKs and their G-protein-coupled receptor signalling targets, the proteolytically activated receptor family (PARs). Funding: Canadian Institutes of Health Research (CIHR) and the Alberta Ride for Dad-Prostate Cancer Fight Foundation

Kallikrein-related peptidase 6 in cerebrospinal fluid of patients with Alzheimer's disease is associated with CSF-TAU, FDG and AMYLOID-PET

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Background:
Alterations in the expression of human kallikrein-related peptidases (KLKs) have been described in patients with Alzheimer's disease (AD). KLK6 was tested for its suitability for distinguishing AD from cognitive controls (NC).

Methods:
As determined by ELISA in cerebrospinal fluid (CSF), KLK6 levels were compared between 32 AD patients stratified to A/T/(N) system with evidence for amyloid pathology and 23 normal controls (NC) with normal AD biomarkers. Associations between KLK6 levels and clinical severity, CSF and positron emission tomography (PET) based AD biomarkers were calculated.

Results:
KLK6 levels were significantly increased in AD and differed significantly between AD A+/T+/N+ and AD A+/T-/N+ or NC with an AUC of 0.922. In AD, KLK6 was significantly and positively associated with CSF pTau and tTau levels as well as positively with regional cerebral glucose metabolism and, if controlled for the ApoE genotype, negatively with regional cerebral amyloid load.

Conclusions:
KLK6 deserves further investigations as a potential biomarker for AD.

Kallikreins and cysteine cathepsins act at opposite poles of thyroid epithelial cells

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Proteolysis happens in different locations in and around thyroid epithelial cells. Cysteine cathepsins have been studied previously, indicating their prominent roles in thyroglobulin processing at the apical pole and within endo-lysosomes of thyroid epithelial cells. Hence, these enzymes are critical in maintaining thyroid physiology for proper thyroid hormone liberation and its release into the blood circulation. In addition, they contribute to extracellular matrix remodeling at the basolateral pole of thyroid follicle cells particularly in cancer. Because kallikreins are an important family of hormonally regulated serine proteases often involved in cancer progression, we extended our studies to address their possible function in thyroid epithelial and carcinoma cell biology. Using human thyroid epithelial cell lines it was found that KLK7 activity is required for their survival in vitro. In contrast, KLK14 activity was determined not to affect normal thyroid epithelial cell survival or proliferation rates in vitro. In addition, regulated secretion of KLK7 and nuclear targeted specific forms of KLK7 were observed in human cell lines representing the most aggressive form of thyroid cancer, namely anaplastic thyroid carcinoma. KLK14 on the other hand appeared to be trafficked along the secretory pathway for secretion and reinternalization by endocytosis in human thyroid epithelial cells. Interestingly, in mouse thyroid tissue in situ, Klk14 activity was detected mainly at the basolateral pole of the epithelial cells constituting the follicles. These results suggested its constitutive secretion from mouse thyroid epithelial cells and a function of Klk14 in extracellular matrix remodeling.

We conclude that the main tasks of the thyroid gland, which are enabled by extracellular proteolysis, are mediated by secreted cysteine cathepsins and kallikreins, respectively. However, the physiological functions of these protease families are realized in thyroid follicles in situ through cleavage of different substrates and at opposite poles of the epithelial cells. While cysteine cathepsins are required for thyroglobulin processing at the apical pole and within the thyroid epithelium, the kallikreins are active at the basolateral pole most likely in extracellular matrix remodeling.

The role of mechanosensitive KLK10 in endothelial biology and atherosclerosis

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Atherosclerosis is a chronic inflammatory disease of the arterial blood vessels that underlies the occurrence of heart attack and ischemic stroke; the leading causes of death worldwide. Interestingly, atherosclerosis preferentially occurs in areas of disturbed blood flow while areas of stable blood flow are protected from developing atherosclerosis by mechanisms involving broad changes in gene expression. We previously developed the mouse partial carotid artery ligation model of atherosclerosis, where the left common carotid artery is ligated and exposed to pro-atherogenic disturbed flow **(d-flow)** while the flow in the right common carotid artery continues to be exposed to atheroprotective stable flow **(s-flow)**. Using this well-established mouse model of flow-induced atherosclerosis and gene array studies, we identified Kallikrein-Related Peptidase 10 (KLK10) as the most flow-sensitive gene in arterial endothelial cells; being upregulated and downregulated by s-flow and d-flow, respectively. Additional studies showed that KLK10 mRNA and protein expression is increased in cultured human endothelial cells by s-flow, while being dramatically reduced under d-flow conditions in vitro as well. We also found that treatment of endothelial cells with recombinant KLK10 or KLK10 plasmids protected against endothelial inflammation as determined by reduced monocyte adhesion and VCAM1 expression in response to the d-flow or TNFα-treatment. Furthermore, recombinant KLK10 injections reduced atherosclerosis development in the partial carotid model of mouse atherosclerosis. In addition, the anti-inflammatory effect of KLK10 was lost when endothelial cells were treated with siRNAs or specific inhibitors of Protease Activated Receptor 1 or 2 (PAR1 or PAR2), indicating the potential role for PAR1/2. However, neither recombinant KLK10 nor plasmid-mediated KLK10 overexpression in endothelial cells was able to cleave PAR1/2 using multiple independent biochemical or cell-based methods. These results suggest that the anti-inflammatory effect of KLK10 is mediated by the mechanisms involving PAR1 and PAR2, but without their direct cleavage. In summary, we found that KLK10 is a novel flow-sensitive protein, which plays an anti-inflammatory and anti-atherogenic role in endothelial cells.

KLK3 activates VEGF-C and VEGF-D

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The lymphatic system is a vessel network that drains liquid from the body's tissues and returns it into the blood. Two members of the Vascular Endothelial Growth Factor (VEGF) family are known to directly stimulate lymphatic vessel growth: VEGF-C and VEGF-D. Both are secreted as inactive proproteins (pro-VEGF-C and pro-VEGF-D) and must be proteolytically cleaved before they can activate their receptors (1, 2).

The enzyme that activates VEGF-C during embryonic development is ADAMTS3, and a compromised ADAMTS3 gene can lead to hereditary lymphedema (3, 4). However, it was unknown whether VEGF-C can be activated by enzymes other than ADAMTS3 (5).

We tested the KLK proteinase family for their abilities to activate VEGF-C and found that VEGF-C can be activated by KLK3/PSA, thereby producing a novel form of mature VEGF-C. We detected physiologically relevant VEGF-C levels in sperm plasma, and VEGF-C activation occurred simultaneously with sperm liquefaction. The activated VEGF-C was able to bind and activate both VEGF receptor-2 (VEGFR-2) and VEGFR-3. We also studied the effect of KLK3 on VEGF-D. While we could see robust activation of VEGF-D by KLK3, we were unable to detect VEGF-D in human sperm plasma (6).

The function of KLK3 for tumor development has been a topic of discussion for decades (7). Both VEGF-C and VEGF-D can act as angiogenic and lymphangiogenic factors. Therefore, our findings provide a link between KLK3 and two hallmarks of tumor progression: the angiogenic and the metastatic switch. The role of VEGF-C and VEGF-D for tumor angiogenesis (8) and lymphatic metastasis (9) has been corroborated by us and others in animal models. However, this relationship has not been equally clear from human correlation studies.

More work is needed to validate which types of tumor, if any, uses KLK3 to activate VEGF-C or VEGF-D. Similarly, it remains to be shown whether VEGF-C in seminal fluid plays any role in reproduction.

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SESSION 05
Therapeutic potential of KLKs (& Drug design)

Thursday 26 September 2019

Session chair: Morley Hollenberg

Jonathan Harris (Australia)
"Design and recombinant production of a bispecific inhibitor of KLK5 and KLK7"

Caitlin Di Paolo (Canada)
"Novel putative kallikrein-7 inhibiting compounds identified through a fluorescence-based enzyme kinetic screen of a targeted library"

Chahrazade El Amri (France)
"Pharmacological modulators of kallikrein-related peptidases: Conception, characterisation and therapeutical applications in inflammation and neurodegeneration"

Design and recombinant production of a bispecific inhibitor of KLK5 and KLK7

Xingchen Chen[1], Simon de Veer[2], Joakim Swedberg[2], Darren Leahy[1], Maria Brattsand[3], Alain Hovnanian[4], Jonathan Harris[1]

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Elegant mouse knockout experiments from Hovnanian, Kasparek and Sedlacek have demonstrated the key roles played by KLK5 and KLK7 in Netherton Syndrome, an important model of inflammatory skin disease and a standalone target for the pharmaceutical industry. Accordingly, there is significant interest in therapeutic inhibition of these enzymes with many campaigns targeting either kallikrein. However, as yet there have been few attempts to block both enzymes simultaneously. Dual K5/K7 inhibition is complicated by the divergent active sites of the two targets with KLK5 being a trypsin-like protease and KLK7 being closer to chymotrypsin. Previously we produced a broad range SFTI-based inhibitor that was capable of inhibiting both enzymes but at the cost of being a general rather than selective inhibitor of serine proteases. Here we describe an engineered, dual-domain kunitz inhibitor with each domain having separate selectivity for KLK5 and KLK7. The protease interaction loops of each domain have been systematically substituted to display amino acid sequences that we had previously shown to be highly selective and efficient substrates for the targets kallikreins. This approach yielded a single inhibitory molecule with inhibitory Kis of 3.7 ± 0.3 nM for KLK5 and 71 ± 7 nM for KLK7. Furthermore, we have established a high-yield expression system allowing facile production in the yeast Pichia pastoris and established the molecular basis of the inhibitor's selectivity for the two kallikrein isoforms. The inhibitor is based on a naturally occurring human protein scaffold and hence unlikely to be immunogenic and represents an excellent candidate for further development as a therapeutic for Netherton syndrome and other inflammatory skin diseases.

Novel putative kallikrein-7 inhibiting compounds identified through a fluorescence-based enzyme kinetic screen of a targeted library

Caitlin Di Paolo[1,2], Ioannis Prassas[2], Eleftherios Diamandis[1,2]

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Tissue kallikrein-related peptidases (KLKs) are a family of serine proteases involved in maintaining normal skin barrier function. Upregulation of kallikrein-related peptidase 5 (KLK5) and kallikrein-related peptidase 7 (KLK7) have been linked to many inflammatory skin disorders characterized by compromised skin barrier function, such as atopic dermatitis and Netherton syndrome. We hypothesize that aberrant skin KLK activity leads to epidermal barrier dysfunction and inhibition of KLKs in the skin through the application of topical inhibitors will alleviate the symptoms of inflammatory skin disorders. A kallikrein targeted library containing drug-like compounds with predicted inhibiting activity towards KLKs was purchased. This targeted library was designed and compiled based on both molecular docking and pharmacophore screening. To identify novel specific inhibitors of KLK5 and KLK7, recombinant active kallikreins were produced and fluorogenic peptide substrates were used to develop a fluorescence-based enzyme kinetic assay. 648 compounds were screened in a 96-well plate format at an initial concentration of 100μM. Those compounds that inhibited the activity of either KLK5 or KLK7 by more than 50% were selected to undergo secondary validation. The screen yielded two compounds that are KLK7 specific inhibitors with IC50 values of 16.7μM and 6.2μM and Ki values of 13.7μM and 1.5μM, respectively. These inhibitors are currently undergoing further characterization for use as topical therapies for inflammatory skin disorders.

Pharmacological modulators of kallikrein-related peptidases: Conception, characterisation and therapeutical applications in inflammation and neurodegeneration

Chahrazade EL AMRI[1]

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The group Molecular and Functional Enzymology at the Biological Adaptation and Aging Research Unit has a wide expertise in protease enzymology since its main research theme is dedicated to the development and mechanistic evaluation of protease inhibitors to validate and optimize drug-like candidates. Namely, projects of the Molecular and Functional Enzymology group are focused on the evaluation of molecules of therapeutic interest or as mechanistic tools (substrates and inhibitors) targeting human proteases (serine proteases, proteasomes, caspases) involved in various pathology contexts (neurodegenerative diseases, cancer, age-associated pathologies...). Several projects in the field of serine protease modulators in particular of kallikrein-related peptidases are in progress within the group with 5 PhD supervised and 4 patents filed since 2012. The topic of the presentation will thus be focused on a sum-up of our main current and past projects in a transdisciplinary perspective ranging from the design of the modulators to their therapeutic evaluation on in vitro disease models **(1-6)**. A special emphasis on kallikrein-related peptidase 6 (KLK6) will be brought with applications in neurodegenerative diseases.

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SESSION 06
Structural and functional aspects of Kallikreins, their substrates and inhibitors

Friday 27 September 2019

Session chair: Jonathan Harris

Peter Goettig (Austria)
"The dynamic structure of KLK8 in activity and inhibition"

Hans Lilja (United States)
"Elucidating the effects of chymotrypsin-like and trypsin-like catalytic activity by prostate specific antigen and human kallikrein 2 on prostate tissue microenvironment, tumorigenesis and blood release"

Niv Papo (Israel)
"Mapping protein selectivity landscapes using multi-target selective screening and next-generation sequencing of combinatorial libraries"

The dynamic structure of KLK8 in activity and inhibition

Peter Goettig[1], Xingchen Chen[2], Viktor Magdolen[3], Hans Brandstetter[1], Jonathan M. Harris[2]

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As one of the few brain expressed KLKs, which also participates in wound healing, human KLK8 or neuropsin exhibits several unusual physiological and biochemical features. Studies from homologous rodent neuropsins identified two natural substrates, which are crucial in the formation of synaptic connections, required for learning. In line with this physiologic function is the observed Ca2+ stimulated and Zn2+ inhibited activity, observed for optimized fluorogenic ACC and chromogenic pNA substrates with the sequence Thr/Trp(P4)-Lys-Leu-Arg(P1). Enzymatic assays with recombinant E. coli expressed KLK8 and its His99Ala variant demonstrated that His99 hampers the binding of even optimal substrates to some extent. In particular the presence of Ala99 reduced the KM considerably and consistently for three investigated substrates. Molecular dynamics explained the distinct P2-P4 subsite cooperativity by the mediation of His99.

Based on the crystal structure, molecular modeling and molecular dynamic studies allowed the straightforward synthesis of cyclic sunflower trypsin inhibitor (SFTI) variants, all comprising 14 residues. However, compared with SFTI variants, inhibiting other KLKs in the picomolar range, an SFTI with a nearly optimal P4-P1 sequence just reached nanomolar inhibition for KLK8. These findings can be explained by the presence of His99, which guards the active site of KLK8 against polypeptidic inhibitors. In line with this finding, KLK8 is the only major skin-derived KLK that is not inhibited by any LEKTI-domain, the major regulators of KLK5, 7 and 14 activities in skin. The special role of His99 in the 99-loop with its unusual shielding function is a fine example of the many variations in the allosteric surface loop network of the trypsin-/chymotrypsin-like serine proteases.

Elucidating the effects of chymotrypsin-like and trypsin-like catalytic activity by prostate specific antigen and human kallikrein 2 on prostate tissue microenvironment, tumorigenesis and blood release

David Ulmert[1], Katharina Braun[2], Amy Tin[2], Anne Byers[2], Kai Beattie[2], Christian von Bodman[2], Dipti Metha[2], Tatsuo Gondo[2], Swaroop Bose[2], Takeshi Hashimoto[2], Sven Wenske[2], Todd Hiricik[2], Howard Scher[2], Peter Scardino[2], Hans Lilja[2]

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Despite being the most widely used biomarker in prostate cancer (PCa), little is known on the pathobiology associated with catalytically active prostate specific antigen (PSA) and human kallikrein-related peptidase 2 (hK2). To study this, we generated genetically engineered mouse models (GEMM) enabling abundant androgen receptor dependent and prostate epithelium specific expression of these proteases. Constitutive conversion of the zymogen-forms was achieved by enabling furin to efficiently cleave and release the activation peptide. As a secondary exploratory aim, we studied whether a switch in substrate specificity by site-specific mutagenesis to render furin-activated PSA with trypsin-like and hK2 with chymotrypsin-like activity, respectively. Responses not dependent of the enzymatic action of hK2 or PSA were evaluated in GEMM engineered to express proteins rendered non-catalytic by site-directed mutagenesis of 2/3 of the catalytic triad residues. Cancer-susceptible transgenic GEMM with prostate specific expression of PSA or hK2 were created by crossing the pb_KLK3 or pb_KLK2 transgenic models with the Hi-MYC, PTEN, and TRAMP models, respectively. The PSA and hK2 expression constructs were also applied for stable transfections of human PCa cell lines. Our evaluations of blood and tissue levels of PSA and hK2 forms showed that only chymotrypsin-like versions of these proteases were released into the blood circulations predominantly as SERPIN-bound complexes. Interestingly, release of chymotrypsin-like PSA and hK2 were irrelevant to cancer; non-catalytic PSA or trypsin-like hK2 were never detected in blood collected from any of the three different cancer susceptible GEMMs or immunodeficient mice inoculated with PCa cell lines stably transfected with our different PSA and hK2 expression constructs. Volumetric evaluations of our models showed that enzymatic activity did not alter PCa growth. However, chymotrypsin-like PSA activity was found to induce activation of metalloproteases associated with several pathologies, including angiogenesis, inflammation and cancer. In summary, our study shows that blood levels of PSA are reflecting an active biological process contingent of its native specific enzymatic activity, which may be associated with pathological processes.

Mapping protein selectivity landscapes using multi-target selective screening and next-generation sequencing of combinatorial libraries

Si Naftaly[1], Itay Cohen[1], Anat Shahar[2], Alexandra Hockla[3], Evette Radisky[3], Niv Papo[1]

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Characterizing the binding selectivity landscape of interacting proteins is crucial both for elucidating the underlying mechanisms of their interaction and for developing selective inhibitors. However, current mapping methods are laborious and cannot provide a sufficiently comprehensive description of the landscape. Here, we introduce a novel and efficient strategy for comprehensively mapping the binding landscape of proteins using a combination of experimental multi-target selective library screening and in silico next-generation sequencing analysis. We map the binding landscape of a non-selective trypsin inhibitor, the amyloid protein precursor inhibitor (APPI), to each of four human serine proteases (kallikrein-6, mesotrypsin, and anionic and cationic trypsins). We then use this map to dissect and improve the affinity and selectivity of APPI variants toward each of the four proteases. Our strategy can be used as a platform for the development of a new generation of target-selective probes and therapeutic agents based on selective protein–protein interactions.

SESSION 07
KLKs in proteolytic (activation) networks

Friday 27 September 2019

Session chair: Peter Goettig

Tomasz Kantyka (Norway)
“Processing of pro-MMPs by KLK14 - a new level of extracellular matrix proteolysis“

Katharina Käfinger (Germany)
“KLK4-mediated cleavage of CXCR3-ligands as an immune evasion mechanism in ovarian cancer“

Leran Zhang (United Kingdom)
“Unlocking the KLK activome in drug-resistant cancer: imaging, biomarkers and target validation using novel activity probes“

Processing of pro-MMPs by KLK14 – a new level of extracellular matrix proteolysis regulation

Katherine Falkowski[1,2], Oliwia Bocheńska[2], Ida B. Thøgersen[3], Ewa Bielecka[1], Magdalena Kalińska[2], Laura Sąsiadek[1,2], Karolina Płaza[2], Andrzej Kozik[2], Jan Enghild[3], Jan Potempa[2,4], Tomasz Kantyka[1,5]

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Secreted kallikreins (KLKs) and matrix metalloproteases (MMPs) are involved in proteolytic turnover of extracellular matrix (ECM) components modulating the pericellular environment in physiological conditions and contributing to pathologies like cancer by excessive degradation of the ECM. Despite pathophysiological importance of these proteases, interconnection between both groups, especially with respect to activation of zymogenic proenzymes remains elusive. MMP activation is essential in severe pathological processes, including inflammation and cancer. Although a membrane-bound subgroup of MMPs (MT-MMPs) was reported to undergo furin dependent activation, an alternative, furin-independent pathway was also described. As human KLK14 co-localizes with MT-MMPs in the pericellular environment, displays trypsin-like activity and has capability to recognize furin-specific-like sequences, it constitutes a candidate enzyme for alternative MMP processing. To test this hypothesis, we have developed a peptide library-based exposition system (CleavEx) to investigate the potential of KLK14 to specifically recognize and cleave proMMP sequences in activation domains. An initial assessment of the library identified a total of ten MMP activation domain sequences recognized and processed by KLK14. The results were validated by both, MS-MS and Edman degradation analyses, thereby confirming the expected cleavage motifs within the MMP propeptides. The analysis revealed that membrane-type (MT) MMPs are likely targeted by KLK14 for activation. Correspondingly, the findings were validated by processing of commercially available proMT-MMPs, namely proMMP14-17 with KLK14. In addition, release of active MT-MMPs was shown using activity assays employing synthetic substrates and gelatin zymography confirming the proper processing of the proMMPs.

In conclusion, our CleavEx-based approach allowed for an unbiased analysis of all 23 human proMMP specific sequences. Membrane type MMPs were the main subgroup of MMPs targeted by KLK14 and their functional activation was validated by full-length proform processing. Therefore, we propose the KLK14-mediated limited processing of MT-MMPs as the alternative pathway for cell-surface-based MT-MMP activation within the ECM.

Keywords: KLK14, MT-MMP, CleavEx, fusion protein, substrate library, limited proteolysis, zymogen activation

KLK4-mediated cleavage of CXCR3-ligands as an immune evasion mechanism in ovarian cancer

Katharina Käfinger[1], Tobias Dreyer [1], Scott Stansfield[2], Judith Clements[2], Holger Bronger[1], Viktor Magdolen[1]

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Tumor-suppressive lymphocytic infiltration of the tumor is an essential requirement for the success of immunological therapy approaches in ovarian cancer. The chemotactic recruitment of tumor-suppressive immune cells is primarily mediated by the CXCR3 chemokine receptor and its ligands CXCL9, CXCL10 and CXCL11. In fact, high expression of CXCR3 ligands was demonstrated to be associated with a high number of tumor-infiltrating lymphocytes and an improved overall survival in ovarian cancer.

In a secretome-wide study, we identified CXCL9 as a target of KLK4, a member of the human kallikrein-related peptidase family. Chemokine degradation could, thus, represent a potent immune evasion mechanism of ovarian cancer. Of note, we and others have previously shown that elevated KLK4 expression in ovarian cancer tumor tissues is associated with poor patient prognosis. We further assessed cleavage of the CXCR3 ligands by KLK4 and analyzed its functional impact in vitro and in vivo. CXCL9, 10 and 11 as well as KLK4 were recombinantly produced and purified. Cleavage assays indicated that CXCL9 and CXCL11 are processed by KLK4 through limited proteolysis with distinct cleavage patterns whereas CXCL10 is completely degraded. The cleavage products' quantities and sequences were precisely characterized using mass spectrometry. Transwell-migration assays showed that all three KLK4-processed chemokines lose their chemotactic ability. In a syngeneic ovarian cancer mouse model we proved that elevated levels of KLK4 resulted in an accelerated tumor growth and significantly shorter overall survival. In a next step we will validate our findings in a breast cancer mouse model and revise the underlying immune evasion mechanism theory using CXCR3-/- mice. The results are intended to delineate immunomodulatory functions of KLK4 potentially defining new therapeutic strategies to improve immune intervention in ovarian cancer.

Unlocking the KLK activome in drug-resistant cancer: imaging, biomarkers and target validation using novel activity probes

Leran Zhang[1,2], Scott Lovell[1], Anna Neodo[1], Chiara Fabbro[1], Edward Tate[1,2]

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KLK's are indicated for their roles in cancer progression and drug resistance through multiple biochemical pathways [1]. However, there is a lack of chemical tools that could monitor KLK activity (which is decoupled from abundance), hindering the validation of KLK's as novel targets or biomarkers.

Our lab has developed a toolbox of activity-based probes (ABP) for KLK2, 3, 6, 7, 8 and 14 that could quantify individual active KLK's in biological samples. This work focuses on the development of probes for KLK6, 7 and 8. We assessed the substrate specificities of KLK's using a Hybrid Combinatorial Substrate Library (HyCoSuL) [2]. Based on this information, selective substrates and activity-based probes were developed. Active KLK's were detected in the supernatant of pancreatic cancer cell lines. Using these probes, we aim to elucidate the role of KLK's as potential targets or biomarkers in pancreatic, brain and prostate cancers. Our quenched activity-based probes are designed for the imaging of active KLK's.

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SESSION 08
HENNER GRAEFF Foundation & E.K. Frey - Werle Foundation session

Friday 27 September 2019

Daniela Loessner (United Kingdom) - Promotion Prize Awardee
"Kallikrein-related peptidases are key elements in the tumour microenvironment"

Henner Graeff Foundation Young Investigator Award - Amiram Sananes (Israel)
"Combinatorial Engineering of APPI Variant with Improved Proteolytic Resistance and KLK6 Selectivity for Cancer Imaging and Therapy"

E.K. Frey - Werle Foundation Young Investigator Award - Srilakshmi Srinivasan (Australia)
"Prostate cancer risk associated Single Nucleotide Polymorphism affects PSA glycosylation and its function"

Kallikrein-related peptidases are key elements in the tumour microenvironment

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A patient's diagnosis and response to treatment is profoundly influenced by the tumour microenvironment enabling cancer cells to grow and survive. Proteases are major regulators of cellular and extracellular processes in cancer. Our work is focused on understanding the roles of kallikrein-related serine peptidases in solid tumours, including ovarian, prostate and pancreatic cancers, and how they can be targeted therapeutically. To recreate the tissue and tumour microenvironment, we developed bioengineered 3D cell culture and orthotopic xenograft approaches and investigated the interplay of kallikreins with tumour-associated and downstream factors and their functions in cancer progression and resistance to chemotherapeutics. We found that kallikreins are part of a proteolytic network in ovarian cancer that modulates cellular processes leading to metastasis and insensitivity to paclitaxel. Simultaneous expression of KLK4, KLK5, KLK6 and KLK7 enhanced ovarian cancer cell proliferation, invasion, spheroid growth, intraperitoneal spread, signalling pathways and pericellular proteolysis in the tumour microenvironment. Upon transcriptome-wide and protein expression analyses and clinical validation using patient-derived cells, we identified several kallikreins and interacting molecules for therapeutic targeting to interfere not only with cancer cell growth but also cancer-promoting factors in the tumour microenvironment. Our findings will help to develop pharmacological inhibitors and to conduct preclinical studies.

Combinatorial Engineering of APPI Variant with Improved Proteolytic Resistance and KLK6 Selectivity for Cancer Imaging and Therapy

Amiram Sananes[1], Itay Cohen[1], Anat Shahar[2], Alexandra Hockla[3], Elena De Vita[4], Aubry K. Miller[4], Evette S. Radisky[3], Niv Papo[1]

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KLK-related peptidase 6 (KLK6) is highly up-regulated in several types of cancers, including breast and ovarian cancers, where its increased activity promotes invasion and metastasis. Inhibitors that specifically target KLK6, however, have not yet been reported, possibly because KLK6 shares a high sequence homology and structural similarity with other serine proteases and resists inhibition by many polypeptide inhibitors. Here, we present a combinatorial approach for engineering specific KLK6 inhibitors via flow cytometry based screening. This was achieved using yeast-displayed mutant library of the human amyloid precursor protein inhibitor domain (APPI), an inhibitor of other serine proteases, such as anionic and cationic trypsins. On the basis of this screening, we generated a four mutant APPI variant (APPI-4M) with a KLK6 inhibition constant (Ki) of 160 pM and a turnover time of 10 days. To the best of our knowledge, APPI-4M is the most potent KLK6 inhibitor reported to date, displaying 146-fold improved affinity, up to 560-fold greater specificity, and 13-fold improved proteolytic stability compared with wild-type APPI (APPI-WT). We further demonstrate that APPI-4M acts as a functional inhibitor in a cell-based model of KLK6-dependent breast cancer invasion. Moreover, we have solved the crystal structures of the APPI-WT/KLK6 and APPI-4M/KLK6 complexes, revealing the structural and mechanistic bases for the improved KLK6 binding and proteolytic resistance of APPI-4M. Finally, we have used the fact that KLK6 is over-expressed in human ovarian cancer cells, and our latest in vivo results demonstrate the ability of fluorescently-labeled APPI-4M to act as a first-in-class imaging agent for detecting ovarian cancer primary tumors, secondary tumors and more importantly, small metastatic sites. We anticipate that APPI-4M will have substantial translational potential as both an imaging and therapeutic agent.

Prostate cancer risk associated Single Nucleotide Polymorphism affects PSA glycosylation and its function

Srilakshmi Srinivasan[1,2], Carson Stephens[1,2], Emily Wilson[3], Janaththani Panchadsaram[1,2], Kerry DeVoss[4], Hannu Koistinen[5], Ulf-Håkan Stenman[5], Practical Consortium[6], Ashley M. Buckle[3], Robert J. Klein[7], Hans Lilja[8,9], Judith Clement[1,2], Jyotsna Batra[1,2]

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Background:
Genetic association studies have reported single nucleotide polymorphisms (SNPs) at Chromosome 19q13.3 to be associated with prostate cancer (PCa) risk. Recently, the rs61752561 SNP (Asp84Asn substitution) in exon-3 of the kallikrein-related peptidase3 (KLK3) gene encoding Prostate-Specific Antigen (PSA), was reported to be strongly associated with PCa risk (P=2.3X10-8). However, biological contribution of the rs61752561 SNP to PCa risk, has not been elucidated.

Methods:
Recombinant PSA proteins were generated to assess the SNP-mediated biochemical changes by stability and substrate activity assays. PC3 cell-PSA overexpression models were established to evaluate the effect of the SNP on PCa pathogenesis. Genotype-specific correlation of the SNP with total PSA (tPSA) levels and free/total (F/T) PSA ratio were determined from serum samples.

Results:
Functional analysis showed that the rs61752561 SNP impacts on PSA stability, structural conformation and creates an extra-glycosylation site. This PSA variant had reduced enzymatic activity and ability to stimulate proliferation and migration of PCa cells. Interestingly, the minor allele is associated with lower tPSA levels and high F/T PSA ratio in serum samples, indicating that the aminoacid substitution may impact PSA immunoreactivity to antibodies used in the clinical immunoassays.

Conclusions:
Our assessment on the biological effects of the rs61752561 showed this SNP to have a potential role in PCa pathogenesis by changing the glycosylation, protein stability, PSA activity and may also impact on the clinically measured F/T PSA ratio. Accounting for these effects on the tPSA and F/T PSA ratio may help to improve the accuracy of the current PSA test.

CLOSING

Friday 27 September 2019

Eleftherios Diamandis (Canada)
"Kallikrein memoirs and future predictions"

Kallikrein memoirs and future predictions

Eleftherios Diamandis[1,2,3]

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My laboratory has been working with kallikreins for well over 20 years. During this period we have seen a lot of developments in the kallikrein field and the expansion of the family to 15 members. In this presentation I will dwell on the activity of my lab in kallikreins over the last 25 years and identify key periods of discovery and the associated players. Then, I will describe what we have promised to do then, and what has been achieved so far. I will also identify key new technologies which may further facilitate the understanding of the physiological and pathobiological roles of kallikreins in disease diagnostics and therapeutics. I will also attempt to predict future developments in the kallikrein field over the next decade.

POSTER SESSION

Wednesday 25 September (16:15 - 17:30)
& Thursday 26 September 2019 (12:15 – 13:30)

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- **Poster 2** - Sabrina Aït Amiri (France) / First-in-class inhibitors of kallikrein-related peptidase 6 promote oligodendrocyte differentiation
- **Poster 3** - Tobias Dreyer (Germany) / KLK4-mediated cleavage of CX3CL1/fractalkine
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- **Poster 6** - Adriana Feliciano Alves Duran (Brazil) / A novel role of a recombinant Kunitz-BPTI-like inhibitor towards KLK5 and KLK7
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- **Poster 15** - Panagiotis Tsiakanikas (Greece) / Identification and study of alternative 3'-untranslated regions (3'-UTRs) of kallikrein-related peptidase (KLK) gene family members using next-generation sequencing (NGS)
- **Poster 16** - George Yousef (Canada) / Exploring protein expression of members of KLK family in prostate cancer
- **Poster 17** - A. F. S. Laureano (Brazil) / Characterization of poloxamer based micelles as drug delivery agents for human tissue kallikrein 7 antibodies generated by phage display

(PO-1) Suomilide and human trypsin selective inhibition to prevent prostate cancer cell invasion

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Trypsin-3 is a highly active protease that has recently been identified as a potential therapeutic target for the reduction of tumor growth and metastasis in prostate, breast and pancreatic cancers. Cyanobacteria are enormous source of natural products, many of which are trypsin inhibitors. We screened extracts of 450 cyanobacteria strains and discovered numerous strains showing selective inhibition towards trypsin-2 and -3. We purified suomilide from Nodularia sphaerocarpa HKVV and found that it potently inhibits human trypsin-2 and trypsin-3 with IC50 values in low nano-molar concentrations, while human trypsin-1 was inhibited at higher concentrations. We also demonstrated that suomilide inhibits invasion of aggressive and metastatic PC-3M prostate cancer cells, while it did not affect cell proliferation. Suomilide has thus been proved to be a highly potent and selective inhibitor of trypsin-3 and prevents cancer cell invasion. This is significant as advanced solid cancers are often fatal only because the tumor cells escape from the primary tumor and form distant metastases and targeting trypsin-3 could potentially reduce tumor growth and metastasis.

(PO-2) First-in-class inhibitors of kallikrein-related peptidase 6 promote oligodendrocyte differentiation

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) that causes severe motor, sensory and cognitive impairment . To date, it is the most common cause of non-traumatic disability in young adults. Kallikrein 6 (KLK6) is a trypsin-like serine protease belonging to the kallikrein-related peptidase family. KLK6 is the most abundant serine protease produced in the CNS where it plays a major role in maintaining homeostasis. This protease is mainly synthetized by oligodendrocytes, the myelin-producing cells of the CNS. KLK6 participates to the turnover of major brain proteins but is also involved in the maintenance of the blood-brain barrier, in neuroinflammation, in myelin dynamics or in oligodendrocyte survival. Due to this central role, deregulation of the expression and/or activity of KLK6 leads to pathological processes, and more particularly to neuroinflammation and CNS demyelination phenomena. KLK6 is thought to be a robust biomarker for MS since this protease is highly increased in the CSF of MS patients. Therefore, KLK6 appears as an innovative therapeutic target for MS. The aim of this study is to identify therapeutic KLK6 inhibitors encompassing the following properties: (i) reversibility, by the implication of KLK6 in the physiology of the CNS, (ii) selectivity, to avoid undesirable side effects. Herein, we identified and optimized original organic inhibitors for KLK6. The designed low molecular weight inhibitors are potent and reversible towards KLK6, their inhibitory potency was also evaluated on proteases involved in the proteolytic network of KLK6. We also provided a detailed structure-activity relationship and dissect out the chemical basis for an optimal inhibition. The hit compounds were devoid of cytotoxic effects on primary cultures of murine cortical neurons and oligodendrocyte precursors (OPCs). Interestingly, some of these hit compounds promote the differentiation of OPCs into mature oligodendrocytes in vitro. In conclusion, these selected compounds constitute promising leads for the development of innovative pro-myelinating therapy.

(PO-3) KLK4-mediated cleavage of CX3CL1/fractalkine

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Distribution and localization of immune cells within the tissue is a major hallmark of tissue homeostasis as well as the innate and adaptive immune system. A molecule which contributes to this allocation is CX3CL1 (fractalkine). CX3CL1 is a chemotactic cytokine which is expressed as a membrane-bound protein. The extracellular chemokine domain provides solid adhesive properties to effector cells expressing the corresponding receptor CX3CR1. The chemokine domain can be proteolytically removed, generating an active soluble CX3CL1 isoform. This soluble form of CX3CL1 is chemotactically active and can guide immune cells from the blood stream to adjacent tissue. Cells expressing the CX3CR1 receptor are NK-cells, macrophages, dendritic cells and T-cells, among others. Proteases known to contribute to the shedding of CX3CL1 are ADAM10 and ADAM17, but also MMP-2 and cathepsin S.

KLK4 is a member of the KLK serine protease family. In normal physiology, it is only expressed in the prostate and, especially, during tooth development. Importantly, KLK4 expression has been shown to be upregulated in various cancer types.

In this study, we elucidated whether KLK4 may also represent a shedding protease for CX3CL1. In vitro cleavage assays with recombinant KLK4 and the extracellular domain of CX3CL1 revealed a 15 kDa cleavage product, as shown by silver staining, corresponding to the CX3CL1 chemokine domain. In vitro cell assays using OV-MZ-6 ovarian cancer cells stably over-expressing KLK4 showed increased secretion of soluble CX3CL1 by applying an ELISA detecting the soluble chemokine domain. This secretion remained stable after inhibition of the two major shedding proteases of CX3CL1, ADAM10 and ADAM17. The chemotactic activity of the KLK4 cleavage product of CX3CL1 is presently tested in cell biological assays.

Shedding of CX3CL1 is an important regulator of the immune cell distribution during disease like inflammation and cancer. We suggest KLK4 as a novel factor within the network of CX3CL1 shedding and immune regulation.

(PO-4) Generation of specific nanobodies targeting kallikrein-related peptidases in alpacas in Chile

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Kallikrein-related peptidases (KLKs) are important serine proteases that have been postulated as important biomarker and players in the cancer progression. Our aim is to develop modern tools for detection of kallikrein pathways by generating nanobodies from alpacas, which display advantages over the generation of antibodies. The Chilean natural barriers and the mountainous landscape allowed the evolution of camelids such as alpacas, llamas, vicuñas, and guanacos, all of them being sources for nanobodies.

In contrast to other mammals, a large proportion of camelid antibodies (up to 80%) belongs to the heavy chain only antibodies (HCAbs), an antibody type lacking the light chains and displaying its antigenic affinity from a single polypeptide sequence. Nanobodies encompass the antigen-specific single variable domain of HCAbs (VHH), are small (approx. 15 kDa), and can easily be recombinantly produced in high quantities. Nanobodies are in general outstandingly soluble and are also applicable as intrabodies which means that they fold correctly in the cytoplasm recognizing intracellular targets with up to sub-nanomolar affinities. Thus, nanobodies have become a useful class of biomolecules for research and various diagnostic and therapeutic applications.

Recently, we have established the procedures for immunization of alpacas, nanobody identification and isolation in our Chilean laboratories. To generate nanobodies directed to KLKs 3, 4, 6, and 15, we immunized alpacas with the respective purified recombinant KLKs, isolated lymphocytes from the alpacas, extracted RNA, amplified the VHH-encoding sequences by PCR, and generated VHH-specific phagemid libraries. Presently, we are isolating selective high-affinity binders directed to the various KLKs and will determine their utility in cancer diagnostics and possibly also therapeutics.

(PO-5) Analyzing the proteolytic network interactions of tissue kallikreins and matrix metalloproteinases using CleavEx libraries

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Kallikreins (KLKs) and matrix metalloproteinases (MMPs) are secretory proteinases known to proteolytically process components of the extracellular matrix (ECM), thus modulating the pericellular environment in physiology and excessively in pathologies, promoting tissue destruction. Nonetheless, the interplay between these enzyme families is not fully elucidated. Interestingly, ECM degradation is prevalent in periodontitis where pathogenic bacterial proteases gingipains, extracellular proteases produced by Porphyromonas gingivalis, are known to induce destructive inflammation of gum tissue, an activity attributed to both, pathogen enzymes and activated host proteinases, yet the direct processing of KLKs by gingipains has not been investigated previously. To assess the possible activation of MMP by KLKs and KLK activation by gingipains, we have developed a peptide library-based exposition system (CleavEx) aiming at investigating the potential of these proteases to cleave proKLK and proMMP activation sequences specifically. KLK13 and KLK14 showed a distinguishable difference when screened with the proMMP CleavEx library. KLK14 showed a predominant specificity towards the membrane-type (MT) MMPs to which KLK13 was only able to recognize MT6-MMP and MMP28. Commercially available proMT-MMPs were analyzed and found to be effectively processed by KLK14 into fully active mature MT-MMPs. In the context of periodontitis, initial assessment showed that gingipains may activate many proKLK sequences. Furthermore, gingipain mediated activation of native proform KLKs into functional active forms was observed. In addition, in vitro studies confirmed the ability of all three gingipains to inactivate serine protease inhibitor of Kazal-type 6 in the presence of saliva.

Although these results are preliminary, it is a step towards understanding the complex protease network in the ECM. The role of gingipains within the epithelium have been shown to promote severe tissue inflammation and destruction, which might impact the normal activation patterns of essential ECM proteases like KLKs. This can theoretically lead to downstream activation of MMPs providing a possible mechanistic link between periodontal disease and tumor development.

(PO-6) A novel role of a recombinant Kunitz-BPTI-like inhibitor towards KLK5 and KLK7

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Background:
rBmTI-A is a recombinant serine protease Kunitz-BPTI-like inhibitor cloned from cDNA sequences that translate to Rhipicephalus (Boophilus) microplus tick serine protease inhibitor. It has two Kunitz domains. The first domain has the amino acid arginine at the P1 position of the reactive site, inhibiting trypsin-like, human plasma kallikreins and plasmin enzymes and the second domain has the amino acid leucine present at the P1 position of the reactive site, inhibiting the human neutrophil elastase. In previous studies, the employment of this inhibitor in an animal model of pulmonary emphysema demonstrated an attenuating effect against the inflammatory process and alveolar destruction normally observed in the disease. According to the specific literature, several kallikreins are present in the respiratory tract and its deregulation is associated with the development of diseases such as asthma, COPD and lung cancer.

Objective:
The aim of the present work is to evaluate the inhibitory potential of the molecule on tissue kallikreins, KLK1, KLK5, KLK6 and KLK7, through computational docking studies and inhibitory activity assays.

Results and Discussion:
The experimental assays revealed that rBmTI-A inhibited all tested KLKs (KLK1, KLK5, KLK6 and KLK7) and the Ki was obtained for KLK5 (93,77 nM) and KLK7 (9,74 nM). Docking experiments suggest that amino acid leucine in P1 site of the second domain is preferred for the interaction between the KLK5 and KLK 7 with rBmTI-A.

Keywords:
KLK, COPD, computational docking, Kunitz-BPTI inhibitors

(PO-7) Novel CRISPR/Cas9 mouse models to study Klk proteolytic networks in vivo: systematic phenotypic screen

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Human KLK gene family consists of 15 members located in a single gene cluster. Majority of KLK genes have orthologs in mouse and mouse models proved to be crucial for understanding pathophysiological roles of KLK proteases in vivo. In our previous works, we have demonstrated that novel genome engineering tools, such as TALENs or CRISPRs can be used for fast and efficient generation of unique animal models that can help us understand roles of individual proteases. Furthermore, programmable nucleases allow easy generation of models simultaneously deficient for multiple KLK genes, which can unravel interaction and redundancies of these proteases in complex proteolytic networks. In order to systematically analyze roles of KLK family members in vivo, we have generated KO models for those KLK genes that have not been previously characterized by systematic phenotyping efforts (IMPC). Here we present novel mouse models deficient for KLK 8, 11, 12, 13, and 15. Initial analysis of these models did not reveal any significant spontaneous phenotypes, possibly due to functional overlap with each other or with other proteases. These results underpin necessity of more advanced mouse models deficient in multiple proteases with similar tissue and substrate specificity, to address their function in a complex in vivo environment.

(PO-8) Impaired lactation in Klk5-/-Klk7-/- deficient female mice

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Klk5 and Klk7 have been previously described as major players in a number of physiological and pathological processes in epidermis. In order to address functions of these proteases in vivo, we have previously generated mouse models deficient for Klk5 and Klk7. To understand interactions and possible functional overlap of both proteases, we have also prepared a mouse model deficient for both proteases simultaneously. While Klk5 and Klk7 single-deficient animals did not show any macroscopic phenotype and bred normally, we observed prominent hyperkeratosis in Klk5-/-Klk7-/- deficient pups. Furthermore, pups obtained from Klk5-/-Klk7-/- mothers showed high mortality rate and only 10% of newborn pups survived to weaning age. The lethal phenotype of newborns did not depend on the genotype and we did not observe any impairments of the mammary glands development. However we found prominent thickening of epithelia at orifices of lactiferous ducts, which might disable efficient lactation. We conclude that cooperative proteolytic activities of both, Klk5 and Klk7 are required for correct formation of milk ducts and efficient milk supply.

(PO-9) Clinical relevance of cysteine-rich intestinal protein 1 (CRIP1) mRNA expression in advanced high-grade serous ovarian cancer

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The small cytosolic zinc-binding protein cysteine-rich intestinal protein 1 (CRIP1; 8.5 kDa) is a member of the LIM/double zinc finger protein family and has been reported to modulate cellular growth, migration, invasion, and differentiation. Aberrant expression of CRIP1 has been detected in several tumor types, such as breast, cervical, colorectal and gastric cancer, displaying either oncogenic or tumor suppressive properties depending on the tumor type. The role of CRIP1 in ovarian cancer is unknown so far.

Previously, we demonstrated that increased levels of several kallikrein-related peptidases, including KLK4, 5, 6, and 7, are associated with poor survival in ovarian cancer. Moreover, simultaneous overexpression of KLK4-7 strikingly upregulated expression of a series of tumor-associated genes, including CRIP1, in ovarian cancer cells. In the present study, CRIP1 mRNA expression levels were assessed by quantitative PCR in a homogeneous cohort encompassing 139 patients with advanced high-grade serous ovarian cancer (FIGO stage III/IV), and the clinical relevance of CRIP1 was investigated. There was no significant association between clinical parameters and CRIP1 mRNA expression levels. In Kaplan Meier analysis, elevated CRIP1 mRNA expression was related with a longer progression-free survival (PFS) in ovarian cancer patients (p=0.019). Moreover, in multivariable COX regression analysis, elevated CRIP1 mRNA expression (HR=0.51, 95% CI=0.27-0.99, p=0.045) remained as an independent prognostic factor for longer PFS, in addition to the clinical factor residual tumor mass (HR=2.38, 95% CI=1.39-4.06, p=0.002).

Altogether, the results of the present study indicate that CRIP1 - in contrast to KLK4-7, which are all related to poor prognosis - represents a favorable prognostic marker in patients with advanced high-grade serous ovarian cancer. Presently, cell biological studies are underway to get more insights into the regulation of CRIP1 gene expression by KLK4-7 and into its tumor biological function.

(PO-10) Tissue factor pathway inhibitor 2 regulates kallikrein-related peptidase 5 functions in lung diseases

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Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disorder that is associated with lung cancer in the third of patients. These two pathologies are essentially due to chronic tobacco smoking, responsible for airway damage and the production of inflammatory mediators and proteases. The imbalance in the protease/antiprotease balance and inflammation process lead to the progressive destruction of lung tissue and alveolar walls observed during COPD exacerbation phases and emphysema. Among proteases the human tissue Kallikrein-related peptidases (KLKs) are serine proteases that could be involved in the extracellular matrix (ECM) degradation and tissue remodelling occuring in tissue repair or tumour progression. We identified the tissue factor pathway inhibitor (TFPI-2), a 32 kDa Kunitz-type serine protease inhibitor as a potent inhibitor of KLK5. As previously described, TFPI-2 is known to be a tumour suppressor gene downregulated in aggressive lung cancer. We demonstrated that TFPI-2 reduces the cell detachment of epithelial cells Beas2B induced by KLK5 but weakly for cancerous cells A549. This loss in cell adhesion to fibronectin, vitronectin and collagen IV is abolished by the addition of TFPI-2. Moreover TFPI-2 limits the cleavage of fibronectin, alpha5beta1 and alpha6beta4 integrins. We also observed that KLK5 increases and improves the cell layer repair and induces clusters with change in cell morphology and acquisition of a mesenchymal phenotype. The modulation of these effects, cell migration and invasion was studied by time lapse videomicroscopy and using modified Boyden chamber assay. All the data suggest that TFPI-2 is a potent regulator of KLK5 activity and could thus modulate the cancer progression and bronchial epithelium repair mediated by KLK-5.

(PO-11) Infection with Herpes Simplex Virus Type 1 alters the expression of KLK6 in vivo and in vitro

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Neuroinflammation is emerging as a central process in many neurological conditions in the Central Nervous System (CNS), either as a causative factor or as a secondary response to nervous insults. This event is coordinated by different processes in which kallikreins-related peptidases (KLKs) might contribute. In fact, KLK6, play a fundamental role in physiological maintain of the brain. In this context, neurotropic pathogens, such as Herpes Simplex Virus Type 1 (HSV-1), can reach the CNS. HSV-1 is characterized by establishing a persistent latent infection in neurons of their hosts for life. Due to its ability to establish latency in neurons, many studies suggest that reiterative viral reactivations at this level could generate accumulative cellular damage, which leads to recurrent neuroinflammatory processes in which KLK6 might contribute. To date, have been not reported if persistent neurotropic HSV-1 infection modulates the expression of KLK6 in the brain. Our results showed that in an in vivo model of HSV-1 infection, KLK6 expression is altered. Specifically, there's an increase of KLK6 protein in the serum of infected animals. Immunohistochemistry analysis of mid-sagittal brain sections of this model showed immunoreactivity against KLK6 in the whole brain, with a high reaction on cerebral cortex cells, as in control as well as infected animals. Indeed, the intensity of the immunoreaction increases significantly in the brains of infected animals respect to controls. Amusingly, increase in KLK6 protein and mRNA expression was also evidenced during an in vitro HSV-1 kinetics of infection using H4 neuroglioma cells and differentiate SHY5Y cells and changes in the expression of KLK6 cannot be elucidated when HSV-1 is U.V. inactivated, suggesting that the changes in KLK6 expression are caused by HSV-1 infection. Accordingly, our results indicate that the protease KLK6 may change its basal expression by injury events during neurological infections. Furthermore, KLK6 could be a useful marker in the study of neuroinflammatory processes, associated with damage caused by neurotrophic pathogens that generate a persistent infection in humans, causing periodic reactivations, such as HSV-1.

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(PO-12) Selective dysregulation of the serine protease KLK14 in stable and exacerbated COPD

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COPD (chronic obstructive pulmonary disease) is characterized by chronic irreversible airflow obstruction with structural changes such as small airway destruction, fibrosis and neutrophilic airway inflammation. Smoking is one of the major risk factors for the development of COPD, although other important risk factors have been also considered. Current findings indicate that pathology and morbidity are driven by dysregulation of protease activity, either through hyperactivity of proteases or deficiency of their antiprotease regulators. This study measured, for the first time, the sputum concentrations of five KLKs (namely, KLK1, KLK5, KLK11, KLK13 and KLK14) in patients with stable COPD (n= 61) compared with those seen in healthy smoking subjects (n = 9). Among KLKs, KLK14 showed a particular evolution since its concentrations fall in sputum from patients with stable COPD. KLK14 concentrations in sputum were significantly associated with disease severity, when assessed by using spirometric values. The more the respiratory constants were degraded, the more the KLK14 concentrations in sputum dropped. To determine if the KLK14 decrease in sputum was due to a reduction in tissue expression, we examined KLK14 expression in the non-tumoral part of lung tissue specimens obtained from patients who underwent lung resection for a lung cancer and suffering or not from COPD. The KLK14 protein levels significantly decreased in tissue specimens from COPD patients (n = 49) when compared with non-COPD smokers (n = 62). This observation indicates a decreased tissue expression of this protease in stable COPD. Exacerbation (AECOPD) was associated with an increase of KLK14 concentrations in sputum; however, the average levels of KLK14 in AECOPD sputum were not restored to the average levels in sputum from non-COPD smokers. This study suggests a role for KLK14 in maintaining lung homeostasis

(PO-13) Gut Bacterial proteases: at the cutting edge of IBD

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While proteases are critical in gastrointestinal physiology, growing evidence has suggested that a dysregulated proteolysis may play a key role in the pathogenesis of several digestive disorders including inflammatory bowel diseases (IBD). Previous studies have reported an increased protease activity in IBD tissues. Such proteases were further suggested to elicit structural and functional changes in the intestinal barrier and promote inflammation. Surprisingly, most of the available knowledge and mechanistic insights in this field were associated to host-produced proteins, while we largely ignore the potential contribution of gut microbial proteases. Many questions remain regarding the distribution and abundance of microbial proteases in the gut community as well as their functional profiling in health and disease. Here, we report the purification and the biochemical characterization of a novel serine protease (Sp1) encoded by human gastro-intestinal tract commensal bacteria. To gain further insights on Sp1 physiological functions, we studied its substrate specificity. Of interest, recombinant Sp1 showed that it has trypsin-like activity. The determination of the biochemical properties of such protease revealed that Sp1 exhibited a highly stable activity at a wide range of temperatures, from 30 to 70°C, with an optimal temperature of 37°C. Analysis of pH behavior showed that Sp1 displayed a broad pH tolerance from 4 to 10, with an optimum pH value of 8. Mechanistically understanding how such protease may influence host responses is a key to identify target proteases allowing the development of new therapies.

(PO-14) Diverse functions of KLK8, KLK13 and KLK14 in the regulation of the wound healing process

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Wound healing is a regulated physiological process that occurs upon tissue damage. It is a process involving inflammation, cell proliferation and remodeling in which various factors are involved. The inflammatory stages entail various cytokines such as IL-6 and IL-8 that stimulate proliferation of epithelial cells and participate in paracrine interplay between keratinocytes and neighboring fibroblasts. Various members of the tissue kallikrein (KLK) family are upregulated in wounded tissue and have been found to participate in this phenomenon both directly and indirectly.

Our research has been able to demonstrate the involvement of KLK8, 13 and 14 in wound healing by studying its effects on primary human skin fibroblasts (HSF). ELISA and real-time PCR experiments have shown that KLK14 is able to induce various inflammatory cytokine production in HSF in a time and concentration dependent manner. Furthermore, KLK14 was found to induce morphological changes in HSF following cell detachment as observed through fluorescence microscopy. These cells were viable due to their ability to re-attach to a new surface. Unlike KLK14, scratch assays demonstrated the ability of KLK8 and 13 to induce HSF migration and culture media transfer experiments indicated that in contrast to KLK8, KLK13 is able to release a soluble, pro-migratory ligand.

These results indicate that KLKs may be involved in various stages of wound healing. Together, kallikreins 8,13 and 14 participate in inflammatory processes, in cell migration and paracrine crosstalk.

(PO-15) Identification and study of alternative 3'-untranslated regions (3'-UTRs) of kallikrein-related peptidase (KLK) gene family members using next-generation sequencing (NGS)

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Background:
Human kallikrein-related peptidases (KLKs) are a subgroup of trypsin and chymotrypsin-like serine peptidases, encoded by the largest contiguous cluster of 15 protease genes and located on chromosome 19 (19q13.4). The majority of KLK pre-mRNAs appear to be alternatively spliced and consequently, the human KLK genes demonstrate over 90 annotated mRNAs so far. KLKs have emerged as promising biomarkers in several types of human malignancies, being aberrantly expressed in cancerous tissues. The aberrant expression of KLKs in human malignancies is often regulated by KLK/miRNA interactions, as many miRNAs have been found to target KLKs and therefore alter their expression levels.

Methods:
In this study, we used an in-house developed assay based on 3'-rapid amplification of cDNA ends (3'-RACE) for the specific amplification of KLK transcripts and next-generation sequencing (NGS) technology to identify novel alternative 3'-UTRs of the human KLKs. For this purpose, total RNA was extracted from 55 human cell lines, followed by first-strand cDNA synthesis, using an oligo-dT-adaptor sequence as primer. In the next step, 3'-RACE was performed for the specific amplification of KLK transcripts. All 3'-RACE products were cleaned-up and used for the library construction. The quality and concentration of the constructed library were determined with quantitative real-time PCR. Finally, NGS was carried out on an Ion PGM™ system, using semiconductor technology. The obtained NGS data were analyzed by bioinformatics algorithms and in-house developed bioinformatics tools we generated for this purpose.

Results and conclusion:
Extensive bioinformatic analysis revealed that KLK transcripts contain new alternative 3'-UTRs, supporting the existence of novel KLK transcripts that are characterized by new alternative 3'-UTRs. Since multiple KLK genes constitute miRNA targets and miRNAs contribute to the regulation of KLK expression in many human malignancies at the post-transcriptional level, the identification of novel 3'-UTRs could be of high significance for the understanding of the regulatory miRNA-KLK network in human malignancies.

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(PO-16) Exploring protein expression of members of KLK family in prostate cancer

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Introduction:
Several studies have shown that Kallikrein (KLK) family members are associated with prostate cancer and are potential cancer biomarkers because of a significant expression differences between normal and tumor tissues. Immunohistochemical staining is an excellent tool to semi-quantitatively assess the protein expression and provide an insight on the subcellular location in each structure of tissue. There have been limited studies of KLK expression using immunohistochemistry in prostate cancer except for KLK3.

Aim:
In this study, we investigated the expression of four KLK family members reported to be associated with prostate cancer; KLK2, KLK4, KLK14, and KLK15 in normal prostate tissues, benign reactive conditions, high grade prostatic intraepithelial neoplasia and acinar adenocarcinoma. We also examined the expression in correlation to the each Gleason grade and its morphologic patterns.
Materials and methods: We used a resection specimens from radical prostatectomy patients with prostatic carcinoma. Immunohistochemical stain was carried out using commercial KLK-specific antibodies and interpreted semi-quantitatively. The intensity and extent of staining were scored and multiplied to obtain a final staining score.

Results:
KLK2 expression was found in the luminal cells of acini, whereas there was no staining in the atrophic glands. The stating pattern in malignant acini was cytoplasmic, and tended to show luminal accentuation.KLK2 stained high-grade intraepithelial neoplasia and Gleason grade 3 glands with similar intensity. KLK4 and KLK14 expression tended to be more intense in higher Gleason grade glands. KLK15 expression was observed in the acinar adenocarcinoma whereas there was negative or weak staining in normal glands.

Conclusion:
The expression intensity of KLK4, KLK14, and KLK15 can be used as biomarkers to distinguish different components of prostate cancer, HGPIN, and adjacent normal tissues. They can provide insight about the pathogenesis of tumor progression and can also serve as prognostic biomarkers.

(PO-17) Characterization of poloxamer based micelles as drug delivery agents for human tissue kallikrein 7 antibodies generated by phage display

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Introduction:
Dysregulation in KLK7 functions is one of the culprits for skin disruption diseases such as psoriasis, atopic dermatitis, Netherton syndrome and others. Phage display is a technique that allows the selection of ligands against, virtually, any antigen; thus KLK7 is a good target to be used in this technique.

Objectives:
To generate recombinant antibodies against KLK7 and create an effective drug delivery system to inhibit KLK7 in the skin.

Materials and methods:
Antibodies against human KLK7 were generated by phage display and expressed in HEK293 cells; the antibody with the lowest IC50 value was submitted to chain shuffling for enhancing specificity. Three new antibodies were selected and encapsulated into poloxamer formulations (F1 = PL407 30%; F2 = PL407 28% + PL403 2%). The formulations were characterized by rheology and dynamic light scattering. After physical-chemical characterization the formulations of poloxamer were tested using in vitro models of skin to determine the dissolution, permeation and liberation in the formulations. The inhibition of KLK7 by the antibodies after those assays was also performed. Cytotoxicity assays were perfomed in Vero cells.

Discussion and results:
Using a scFv gene library it was possible to select one antibody with IC50=2.3 nM. After affinity maturation three new antibodies were generated, with lower IC50 (0.52; 0.81 and 0.95 nM). The micellar hydrodynamic diameters ranged from 30.7 to 36.4 nm for F1 and F2, showing higher micellar dimensions for F2 after antibodies' incorporation. The micellar transition temperature was observed at 12.5ºC and 14.8°C for F1 and F2 samples, respectively. Rheological analysis showed similar sol-gel transition temperatures for all systems. Dissolution assays showed that time does not influence in the release. 80 and 60% were the maximum release rates for F1 and F2, respectively. The lowest inhibition values were 0.7 (F1) and 1.1 (F2) nM. Permeation assays confirmed the antibodies' capacity to cross the skin-like membrane maintaining the inhibitory capacity. Cytotoxicity assays showed antibodies' capacity of modulating cell growth.

Conclusion:
A drug delivery system based on hydrogels to antibodies against human KLK7 was created.
Keywords: drug delivery, hydrogels, KLK7, phage display

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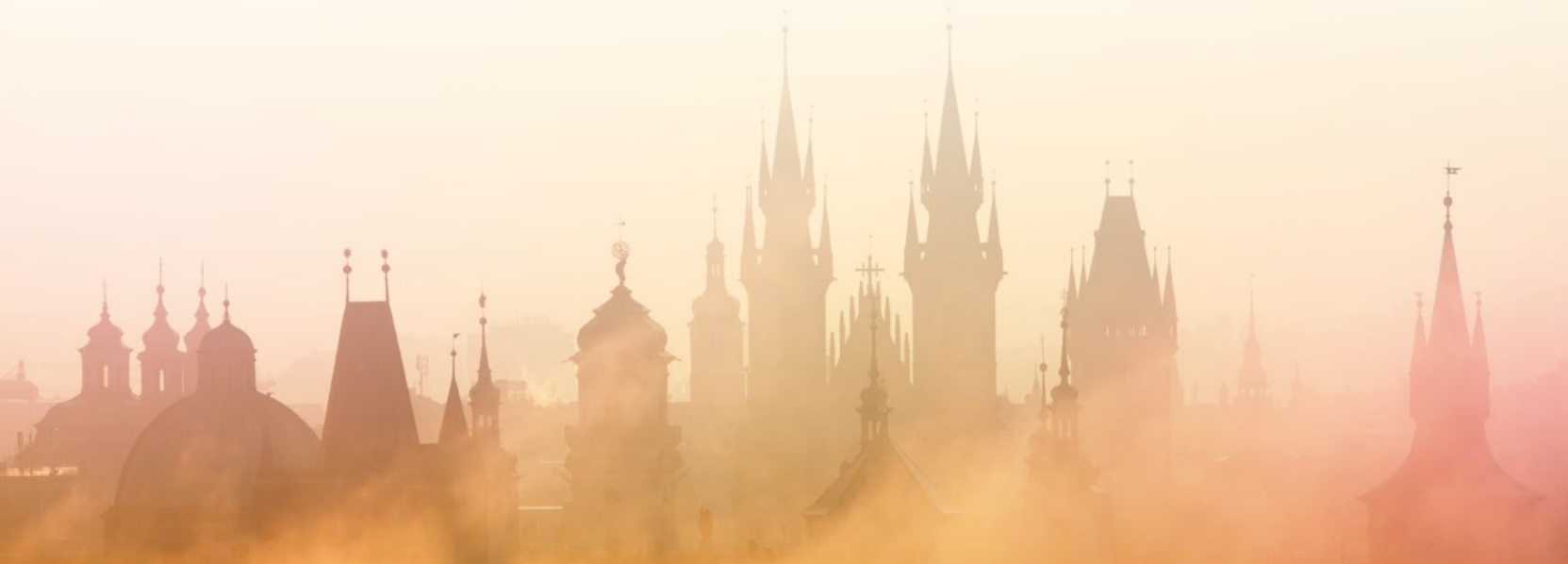

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